SUBMICROSCOPIC MORPHOLOGY OF PROTOPLASM AND ITS DERIVATIVES

by

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FOREWORD

This monograph is the second edition of my „Submikroskopische Morphologie des Protoplasmas und seiner Derivate" published in 1938 by Gebrüder Bornträger Berlin. War and post-war conditions made it impossible to republish this book in German. For that reason I was glad to accept the offer of the Elsevier Publishing Company, Inc. Amsterdam to translate the manuscript of the second edition into English.

The aim of the first edition was to introduce Submicroscopic Morphology as a new branch of General Morphology. As, in 1938, the electron microscope had not yet become an instrument of biological research, that introduction was based on the results of indirect methods of investigation (macromolecular chemistry, double refraction, dichroism, X-ray diffraction etc.), which made it possible to provide evidence of the arrangement of submicroscopic elements. In general one indirect method alone will not yield an unequivocal postulate as to a structure invisible in the ordinary microscope. But a combination of several such methods made it possible to exclude certain possibilities. Submicroscopic Morphology, therefore, was an exciting and inspiring field of trial and error for morphologists interested in Biophysics.

Since then the electron microscope has made it feasible to photograph submicroscopic structures and to check the results of the indirect methods. It is a great satisfaction for the pioneers of Submicroscopic Morphology to know that their postulates as to the structures of gels, fibres etc. were right. On the other hand, our science has lost one of its attractive charms; we no longer have the satisfaction of inventing new methods of research and seeking the particular structural arrangement which agrees with the results given by all the available indirect methods and therefore must correspond to the real invisible structure. This romance of discovery has given place to the technical problem of obtaining objects thin enough to get the best possible image in the electron microscope.

This second edition had to face the fact that Submicroscopic Morphology has become generally accepted as an important branch of biological sciences. The morphologists who did not trust the indirect methods willingly accept the results of electron microscopy, although electron optics are even more complicated than those of polarised light or X-rays. But the objectively visible image has always been the foundation of Morphology, and therefore the research in Submicroscopic Morphology will be governed henceforth by the remarkable invention of the electron microscope.

All this notwithstanding, this new edition is not centred on the electron microscope; the old indirect methods, which were just as new and stimulating in their time, are treated as equivalent means of research. The polarising microscope and even the X-ray camera are more accessible to the average biologist, who is interested

in fine-structures, than the expensive electron microscope. There are several excellent monographs on electron microscopy and there is no need for yet another. But this scholarschip often ignores the results of other methods in Submicroscopic Morphology. In the first rush of publishing electron micrographs, many images were produced which would have been discarded as pictures of artifacts, if a thorough knowledge of the results of indirect methods had existed. Where there is doubt as to the accuracy of an electron micrograph, the results of the indirect methods must be taken into consideration. Any discrepancies between the interpretation of the results of indirect methods and those of the electron micrograph must be cleared up, before a submicroscopic structure may be regarded as definitely established.

This book is written, not for specialists, but for students who are attracted towards this interesting field of research. It is an outline which does not attempt to give full details. These must be sought in the literature quoted. Unfortunately printing difficulties have delayed publication, so that most of what has appeared during 1947, as well as the results of the 6th International Congress of Experimental Cytology in Stockholm (1947) are not included. Nevertheless, the extensive literature published on this subject since 1938 has been taken into account, and shows the enormous development of Submicroscopic Morphology during this short period.

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INTRODUCTION

THE DOMAINS OF MORPHOLOGY

Dass ich erkenne, was die Welt
Im Innersten zusammenhält.
GOETHE's *Faust*

Morphological biology comprises the knowledge of organs[1] (anatomy in medicine, organography according to GOEBEL), of tissues (histology) and of cells (cytology). Together these domains form a hierarchic system, as they describe units of diminishing size in the above order. The different domains are defined by the concepts organ, tissue, and cell. They can also be characterized, however, by the expedients which are used to make the different units visible, since each of the sciences mentioned makes use of different instruments of observation. The organographer observes with the naked eye or with the magnifying glass, the histologist with the ordinary microscope and the cytologist with the more refined immersion, phase-contrast[2] or even ultraviolet microscopes. Accordingly, the range of research of organography is in general limited by the resolving power of the eye, the domain of cytology by the resolving power of the microscope (Fig. 1). In biology, all that can be described with the aid of these means of observation is referred to as *morphology*.

The hierarchy of morphology, however, goes beyond the resolving power of the microscope. The persistent, I might almost say the heroic struggle with which the resolving power of the microscope has been increased (ABBE; KÖHLER) gives evidence in this respect. Fig. 1 shows how the microscopic domain was widened step by step by advances in the theory and technique of optics until at about 0.1 μ the absolute limit to a true image of the object drawn by the wave-length of the ultraviolet light was reached. Until recently, morphology was forced to remain at this limit. We have great admiration for the numerous cytologists who have worked in the limiting regions of the optical resolving power of the immersion microscope, pursuing ever finer structures, with ineffable devotion and utter disregard for their eyesight. However, if one remembers their labour and its limited prospects of success (since the actual ultrastructure of the protoplasm cannot be obtained with any certainty by microscopic means) it is remarkable how few biologists have drawn the obvious conclusion from the theory on the limit of microscopic images and have turned their attention to indirect methods of research.

The resolving limit of the microscope is like the shore of a mysterious mountain lake. On land the geo-morphologist can easily recognize all particulars of shape and size, he can measure and photograph them. If, however, he wants to study the mor-

[1] In this connection, "organ" is to be understood in the morphological sense as part of an organism. If the physiological definition "organ = instrument" is based on specific functions, then of course also single tissues, especially cells or even parts of cells can act as "organs".

[2] ZERNIKE, F., 1946.

Fig. 1

THE DOMAINS OF MORPHOLOGY

Organography			Histology	Cytology	Micellar studies		Molecules		Atoms
1 cm	1 mm			1 μ			1 mμ	1 Å	
1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8} cm	

resolving limits:

eye 0.07-0.08 mm (at distance of 25 cm)

microscope (dry system) ca. 0.5μ

immersion microscope 0.3-0.4μ

ultraviolet microscope 0.15-0.2μ

limit of visibility of gold particles in the ultramicroscope 6 mμ

electron microscope (1943) 2.5 mμ

← macroscopic → | ← microscopic → | ← submicroscopic → | ← amicroscopic →

phology of the bed of the lake, he will derive no benefit from a study of the shoreline, however carefully this may be done. Nor will it serve his purpose if he tries to look at the bottom through the water above it. He must not cling stubbornly to the rocks at the shore but must make himself free from the land. He must "swim", and from the surface of the water must find out indirectly with a plummet how the bed is shaped. Each fathoming provides him with a point, and the profile of the bed can then be constructed by interpolation.

Until recent times, in the submicroscopic domain which lies beyond the microscopic limit, the situation was completely analogous. Views on submicroscopic morphology could be obtained only by sounding, i.e., by indirect means, and the invisible shapes and sizes could be deduced only from a combination of the various methods of research.

The discovery of the electron microscope after 1938 suddenly brought the submicroscopic regions within reach. By means of electron rays the resolving power has been increased a hundred fold in one sudden leap. The surface of the water in the lake to be studied has, so to speak, been reduced to a much lower level. The precipices and gullies which thus far had been hidden have become easily accessible to the investigator, who is now equipped with the means to move about in this difficult province. Submicroscopic morphology has accordingly lost something of its mysterious charm. Its secrets need no longer be unravelled exclusively by a combination of partial solutions obtained indirectly, as still has to be done in the study of the constitution of organic molecules in structural chemistry. There does now exist a means to check directly the conceptions developed so far. The objective microphotographs given by the electron microscope have made submicroscopic morphology very popular in biology, whereas formerly it had been left to a few biologists with a knowledge of physics.

However the electron microscope cannot replace the indirect methods which so

far had been applied successfully. To begin with, there exist many objects which for technical reasons cannot yet be imaged in the electron microscope, and further, the irradiation with electrons represents a bombardment which, compared with the irradiation with light, involves incomparably greater energies, which are apt to destroy organic structures within very short times. The electron-optical images of biological objects should therefore be considered critically. They must be compared with the partial results obtained from the indirect methods, and in cases of contradiction it must be made clear on which side the error lies. In this way it has been possible in some cases to show that the electron microscope had produced spurious effects. Electron microscopy should not, therefore, supersede the methods applied so far, such as polarisation microscopy or X-ray analysis, but the new direct method and the efficacious indirect methods must be used jointly, each acting as a check on the other, in the exploration of the submicroscopic domain.

The history of this important science will soon be able to celebrate its first centenary (NÄGELI, 1858). Only in the last twenty years has there been a continuous development of, and increasing interest in this field of research. For AMBRONN, who devoted his whole life to this branch of science and who published his fundamental researches on the rod-like nature of the structural elements of gels in 1916–17, had to carry out his work, according to his own statement, "excluded from publicity", and until his death in 1927 he considered that his was the voice of a biologist crying in the wilderness. The general lack of interest in submicroscopic problems was doubtless due to the following. Colloid chemistry had developed into a general doctrine of dispersoids. The discovery of the ultramicroscope (SIEDENTOPF and ZSIGMONDY) had suddenly given rise to a considerable extension of the submicroscopic morphology of sols. With great enthusiasm biology made itself master of the new method, but with disappointment ascertained that nearly all important biological objects: cytoplasm, nuclei, plastides, cell-walls, etc. are "optically empty". We know now that this is not only due to the close packing or the hydrophilic nature of the hypothetical particles, but above all to the fact that we have to deal with anisodiametric structural elements, which are already invisible in the ultramicroscope if only one of their dimensions is amicroscopic, even if such structural elements accumulate in loose meshworks of submicroscopic or even microscopic dimensions. This indicates that biological gels do not at all represent dispersions in the classical colloid chemical sense (see Table II). The failure of the ultramicroscope led one to conclude that these objects did not possess a submicroscopic structure.

In the meantime structural chemistry has developed amicroscopic molecular morphology. X-ray analysis has provided us with exact data on the mutual position and distances of the atoms and groups of atoms in organic molecules, and this greatly added to our knowledge of stereochemistry. Nowadays we know, not only the structural formula of many compounds, but also with astounding accuracy their entire morphological structure.

From the molecular region, the elucidation of the constitution of high polymers has already advanced into the submicroscopic region as a new branch of structural chemistry. In the case of polysaccharides and polypeptides, for instance, it shows that thousands of similar structural elements can be united to gigantic chain molecules which sometimes even reach microscopical lengths. STAUDINGER to whom we owe this knowledge designates this new kind of study as macromolecular chemistry.

This might lead one to believe that the link between cytological and molecular morphology has been forged and that, consequently, a special submicroscopic morphology would become superfluous. This, however, is by no means true, for, the high polymer chains can arrange themselves in more or less regular lattices which in their turn cluster together to form porous structures, interwoven with numerous capillary spaces of various sizes. Or again, they form loose meshworks with a totally different degree of order. Besides chains, there may occur flake-like high polymers, thus allowing for a great many possible arrangements of the submicroscopic elements. Consequently, in addition to the problems of constitution in macromolecular chemistry, there exist morphological problems of a special kind, the description of which can best be characterized as the *morphological study of fine-structure*. In biology this nomenclature is synonymous with the study of *micellar systems* (FREY 1928a), provided the new definition on p. 57 is taken into account.

In Fig. 1 the lower boundary line of the morphological domain of the fine-structures has been drawn arbitrarily at the limit of visibility of the smallest gold particles in the ultramicroscope. The resolving power of the electron microscope, which can still be improved, lies for the present within the same range. This serves to show that the order of magnitude of our field of research coincides with that of classical colloid chemistry. In contrast with the isolated dispersed particles, however, the colloid dimensions do not refer to all three directions in space but, in the case of rodshaped elements to two dimensions only, or even to only one with flake-like submicroscopic elements, which may be clustered to form complicated systems.

TABLE I

MORPHOLOGY

Morphological hierarchy		Instruments of research	Scales	Order of magnitude
Organs	Organography	Eye, magnif. glass	mm scale	> 0.1 mm
Tissues	Histology	Microscope	Micrometer	> 1 μ
Cells	Cytology	Immersion and ultraviolet microscope	Wave-lengths of light	> 0.1 μ
Fine-structure	Micellar studies	Polarisation microscope	Fraction of wave-length	< 0.1 μ
Molecule structure	Structural chemistry	X-rays	Wave-length of X-rays	> 1 Å
Atom structure	Electron theory	Electron rays	Wave-length of electron-rays	< 0.1 Å

According to Table I the domain of fine-structures forms a link between our present knowledge of cytological and molecular morphology. We must therefore attempt to penetrate into the study of micellar systems from these two known sides. Starting from the region of visible structures, we must resort to our knowledge of *phases*, while on the other, molecular, side we should apply our knowledge of *crystal structure*. Both these theories cover morphological domains which fall outside the hierarchy given in Table I. There is no upper limit to the dimensions of phases, although there does exist a lower limit which must be discussed thoroughly. Simi-

larly, there exists no upper limit on theoretical grounds to the regular arrangement of atoms and molecules in crystal lattices. So that we can use these abstract sciences, which are less sensitive to dimensions, as an introduction to the study of fine-structures.

Morphology is not an ultimate goal of science, but it represents one of its most important foundations. No physical problem can be attacked without first defining accurately the mutual positions of the various points in the system to be investigated. It is only after this that time can be introduced as a variable, to pass on from static to dynamic considerations. Just so in biology. All *physiological*[1] research, being concerned essentially with changes in the course of time, pre-supposes a complete knowledge of morphology. The relations between the various organs and tissues can be studied in their dependence on time only, if their spatial arrangement has been ascertained with accuracy. This explains the tremendous flight which the physiology of the human body has taken in connection with the development of anatomy and histology.

Passing from the total organism to the elementary organism of the cell, we must expect similar relations. If, therefore, we want to study the physiology of cells successfully, we must know its morphology as thoroughly as that of the total organism. The invisible texture of the cell, however, which is the object of fine-structure or micellar morphology is still in its infancy. The difficulties in this field of research are great and at present we still do not know how far we shall be able to proceed. Each new gain in this direction, however, will not only augment the archives of the descriptive science of nature, but also be to the advantage of physiology, and will in the end satisfy our thirst for knowledge.

[1] *Physiology* is the science of *events* and *processes* in living organisms. Both these expressions clearly indicate that time is involved, i.e., they show the dynamic character of physiology. *Biomorphology* and *biochemistry*, on the other hand, are not concerned with time; the one describes the spatial arrangement and the other the properties of organic matter. It is only when time begins to play a part that morphology becomes *physiology of development* and biochemistry becomes *physiology of metabolism* which, combined, give general physiology, taking into account all variable quantities: space, matter and time, which are accessible to our tools of research. In view of this, we fail to see why the attribute "dynamic" is nowadays added so readily to the branches of knowledge which describe biology. As definitely inconsistent I must consider a combination such as "dynamic morphology", because according to definition morphology can always only describe or explain given spatial arrangements, and as soon as *changes* in spatial arrangement are considered we enter the domain of physiology.

I. FUNDAMENTALS OF SUBMICROSCOPIC MORPHOLOGY

"Le cytoplasme proprement dit se présente sur le vivant comme une substance colloïdale homogène, translucide, optiquement vide à l'ultramicroscope . . ."

GUILLIERMOND, MANGENOT et PLANTEFOL
(1933, p. 386)

§ 1. ORGANISATION OF SOLS

a. *Invisible Particles*

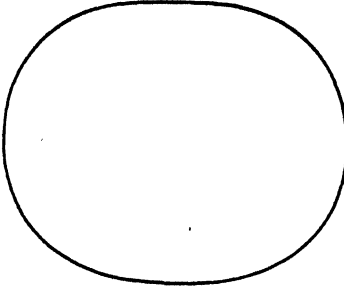
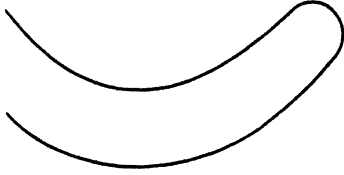
Since GRAHAM (1861) has shown that the pseudo-solutions which impede filtration and which nowadays we call *sols* contain relatively large particles which diffuse slowly, the nature of these invisible particles has been explored in all directions by colloid chemistry.

Demonstration and shape of the particles. Numerous methods have been worked out to distinguish and to separate the originally hypothetical submicroscopic colloid particles from the amicroscopic molecules. By means of *dialysis* the amicroscopic particles can be made to permeate through a semi-permeable membrane (parchment) through which the colloid particles cannot follow (GRAHAM, 1862). This method has since been developed into the *ultrafiltration,* in which sols are pressed through filters with submicroscopic pores (collodion films of various compactnesses) and in this way are split up into fractions of different particle sizes. Further, since most colloid particles carry an electric charge or can be charged by a change in the acidity of the surroundings, they can be made to migrate in an electric field to the anode or to the cathode according to their charge, and it is possible in this way to concentrate them by *electrophoresis.*

None of these methods of indirect particle identification, however, is quite so convincing as *ultramicroscopy* which makes the particles visible (SIEDENTOPF and ZSIGMONDY, 1903). The ultramicroscope, admittedly, does not give a true image of the colloid particles, because its resolving power does not surpass that of the ordinary microscope. Rather, it only succeeds in showing the existence of submicroscopic particles. The possibility of ultramicroscopic demonstration is based on the fact that light incident upon submicroscopic particles is scattered in all directions. In this way every particle becomes like a radiant particle of dust (compare the dust particles in a beam of sunlight), so that the path of a beam of light in a sol is clearly traced (TYNDALL scattering). The lighted sphere surrounding such a dust particle is much larger than the scattering particle itself, and an image of it can be obtained in the microscope if the distance between the colloid particles is not too small. As the objective of the microscope gives an image of planes only, optical cross-sections of the lighted spheres are imaged in the form of *deflection discs.* Since the particles in the sol take part in the

Brownian movement, these scintillating "deflection discs" oscillate vividly in an irregular manner. It is an impressive sight to watch these luminous spots which, in untiring movement, contrast like bright stars with the pitch-dark background.

It cannot be determined how far the size of the "deflection discs" exceeds that of the particles from which they originate. Nor can anything be said about the exact shape of the particles. All the same, the ultramicroscope allows of conclusions about their circumference in those cases where they deviate strongly from the spherical form. Non-spherical particles can be oriented in a field of flow. In that case they scintillate to different extents according as the incident ultramicroscopic irradiation is parallel or perpendicular to the direction of flow; they show what is called azimuth-effect. If the light falls upon the small endplane of submicroscopic rods, they scatter much less than with side ways irradiation. From such differences in intensity of the "deflection discs" in dependence on the direction of the incident beam, the rod-like shape of the particles can be inferred.

Anisodiametric particles usually are birefringent. As they are oriented in a field of flow, sols containing such colloid particles become optically anisotropic in a velocity gradient (FREUNDLICH, STAPELFELDT, and ZOCHER, 1924). Long rods are oriented at lower rates of shear than shorter ones (SIGNER and GROSS, 1933). From measurements of the birefringence of flow, conclusions can therefore be drawn regarding the ratio between length and thickness.

Size of the particles. With the aid of the methods mentioned one can already obtain a clear picture of the world of submicroscopic particles. But colloid chemistry was not content with these qualitative conclusions; it tried to obtain quantitative data about the size of the particles. A certain hold was provided by ultrafiltration, but apart from that, much more accurate methods were available.

If the number of particles in unit volume is determined in the ultramicroscope, the particle size can be calculated from the concentration of the sol. Moreover, there exist mathematical relations between Brownian movement (EINSTEIN's formula), velocity of sedimentation (STOKES' formula) or diffusion on the one hand, and particle size on the other hand. These allow of a determination of the diameter of spherical colloid particles. In these investigations the ultramicroscope plays an important part (ZSIGMONDY, 1925), since in counting or in measuring the Brownian movement, the particles have to be observed. In many cases, however, the colloid particles cannot be established ultramicroscopically, not only because their dimensions often are too small but, above all, because their refractive power often is only slightly different from that of the dispersing medium, so that light scattering is insufficient. This usually applies to biological sols with their organic colloid particles, which means that in the case of these sols the limit of visibility in the ultramicroscope is reached incomparably sooner than in the case of inorganic sols (compare Fig. 1).

The method of sedimentation is free from this difficulty, because the change in concentration of the solution as a result of the sedimented particles can be determined by analytic means or, still more simply, by the change in refractive index. Moreover, the sedimentation velocity can be increased at will by applying stronger centrifugal forces. The *ultracentrifuge* which was developed by SVEDBERG (1938a) into an instrument of the highest accuracy and unbelievable capacity (centrifugal fields which are 750000 times that of the gravitational field!) allows of the determination of particle weights down to amicroscopic molecules.

By the various methods mentioned it has been possible to obtain so deep an

Fig. 2

PARTICLE SIZES (PARTLY FROM STANLEY, 1938a, b)

	Mol. weight	⌀ in mμ	Length (mμ)
1. Red globules		7500	
2. *Bacterium coli*		3000	6000
3. *Bacterium prodigiosum*		750	1000
4. *Treponema pallidum*		200	18000
5. Small-pox virus	$2300 \cdot 10^6$	175	
6. Chicken plague virus.	$300 \cdot 10^6$	90	
7. *Megatherium bacteriophagus*	$23 \cdot 10^6$	38	
8. Yellow fever virus	$4.3 \cdot 10^6$	22	
9. Gene, calcul. accord. to MULLER (1935)	$33 \cdot 10^6$	20	125
10. Tobacco mosaic virus	$43 \cdot 10^6$	12.3	430
11. Foot and mouth virus	$0.4 \cdot 10^6$	10	
12. Glycogene, according to HUSEMANN and RUSKA (1940)	$1.5 \cdot 10^6$	10	
13. Haemocyanin from *Octopus*	$2.8 \cdot 10^6$	8	64
14. Smallest ultramicroscopically visible gold particles, according to ZSIGMONDY (1925)	$2.7 \cdot 10^6$	6	
15. Horse haemoglobin	$69 \cdot 10^3$	5.5	
16. Ovalbumin (SVEDBERG 1930)	$40 \cdot 10^3$	4.3	
17. Saccharose	342	0.5	1.0
18. Hydrogen molecule	2	0.2	

measuring scale: 100 mμ

insight into the morphology (size and shape of submicroscopic particles), that the task left to the *electron microscope* in reality was no more than to confirm by direct images the results obtained by indirect means.

Fig. 2 represents a series of submicroscopic particles of biological importance,

which allow of a comparison with the microscopic and amicroscopic regions. The size and shape of the particles have been determined by the methods mentioned and in many cases also by the electron microscope. It is seen that there is a continuous transition from the lifeless amicroscopic molecules to the living cells at the limit of microscopic visibility. The smallest particles which exhibit phenomena of life (self-multiplication) are in the submicroscopic region. Theoretical biology, being concerned with the definition and the essence of life, must therefore deal thoroughly with our new branch of morphology. On the other hand these colloid particles often give the impression of consisting of uniform, chemically well-defined substances, and the biochemist attributes molecular weights to them which, according to the size of the particles, may assume phantastically large values.

b. *Homogeneity*

Real solutions containing amicroscopic particles are designated as uniform or *homogeneous* from a physico-chemical point of view. Sols, however, are not considered uniform, they are *heterogeneous*. The concept of homogeneity applied here is essentially different from the optical homogeneity which plays such an important part in microscopy. A medium is optically homogeneous when its constituent parts have the same refractive index, so that it is impossible to establish their boundary line by means of light.

Physico-chemical homogeneity, however, requires that two parts taken from the object shall be identical not only in their behaviour towards light, but also in all other properties. This will be the case if the particles are similarly arranged throughout the whole object (Fig. 3–7).

Several homogeneous arrangements of particles are possible. The structural elements can be arranged irregularly, like the molecules of a liquid or gas. The distances between the particles are not all equal, but if we proceed through the mass along a straight line, the average distance found will be constant, and equal volume elements will on the average contain an equal number of particles. Such arrangements are called *statistically homogeneous* in contrast to the distribution of the atoms in a crystal, which are arranged in a certain pattern. As all distances in a given direction are identical, this is called a lattice arrangement. The spacings can be equal in three directions which are mutually perpendicular; in that case the lattice arrangement is isotropic (Fig. 4). Or else, the spacings are different in different directions, in which case the lattice arrangement is anisotropic (Fig. 5). The homogeneous lattice arrangement has in common with the statistically homogeneous arrangement that equal volumes contain an equal number of particles. With anisotropic arrangements it is not sufficient to compare volumes of equal size; they must also have the same orientation. For, if from Fig. 5 instead of circles we draw two congruent rectangles with different orientations, the properties of one of these rectangles will be different from those of the other on account of the different distribution of lattice points with respect to the length of the rectangle (the linear thermal expansion of the long side of the two rectangles, for instance, will be different). The necessity to take orientation into account becomes particularly apparent if polar particles such as, for instance, water molecules are arranged homogeneously. Fig. 5 shows such particles in a statistically homogeneous distribution and Fig. 6 gives an example of an arrangement in a lattice which has identical spacings in three directions.

From these considerations we derive the following definition of homogeneity:

an object is homogeneous if equal and equally oriented parts, taken arbitrarily from the object, possess the same internal structure. This implies that all the parts thus compared have the same physical and chemical properties.

An important condition in these considerations is the order of magnitude of the volumes to be compared. Physico-chemical homogeneity requires that they shall be of *submicroscopic dimensions.* The internal structure, therefore, refers to the arrangement of atoms, ions, and molecules, which in Fig. 3–7 have been indicated by points or arrows.

It follows from this definition that sols cannot be homogeneous. For, if in a sol we consider submicroscopic volumes of sufficiently small size, the one may contain a colloid particle, while the other may merely contain the solvent, i.e., the *dispersing medium*. In contrast to sols, not only all pure substances, whether in the solid, liquid or gaseous state (Fig. 8–10) but also real solutions are homogeneous, provided the solute consists of amicroscopic particles (Fig. 11). If, however, differences in the concentration, for instance concentration gradients, occur in the solution, it is heterogeneous. Similarly, either homogeneous or heterogeneous mixed crystals can originate from a solution or melt, according as the two components can unite to a crystal lattice in a regular or in an irregular distribution (Fig. 12 and 13).

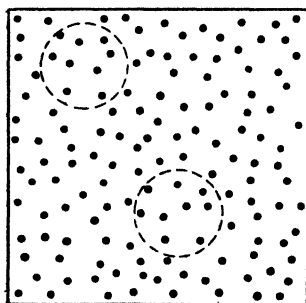
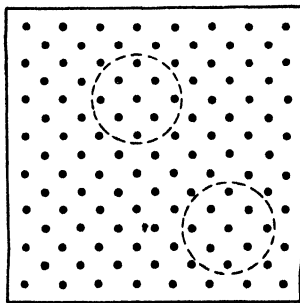
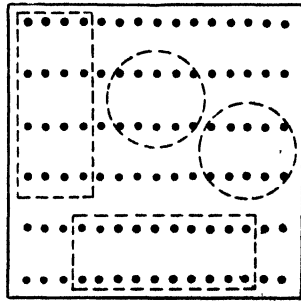
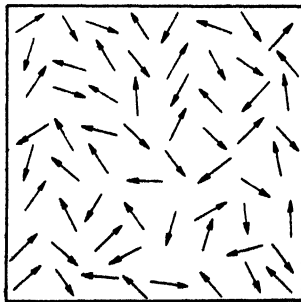
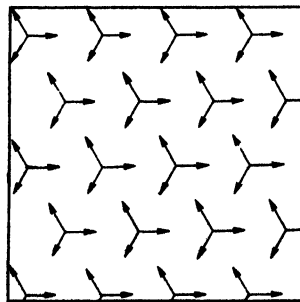
Colloid solution having been recognized as heterogeneous, the question further arises whether the colloid particles themselves may be considered as homogeneous.

Homogeneous arrangements

Fig. 3. Statistically homogeneous distribution – Fig. 4. Homogeneous isotropic lattice – Fig. 5. Homogeneous anisotropic lattice – Fig. 6. Statistically homogeneous distribution of polar particles – Fig. 7. Homogeneous lattice arrangement of polar particles.

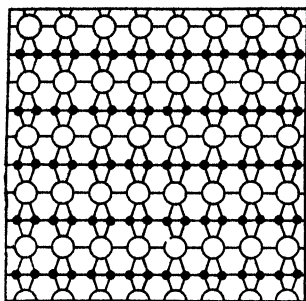
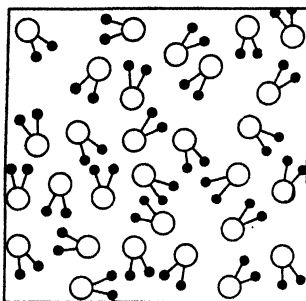
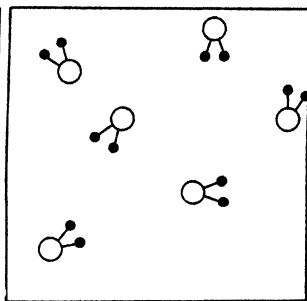



Fig. 8 Fig. 9 Fig. 10

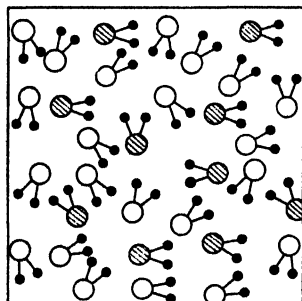
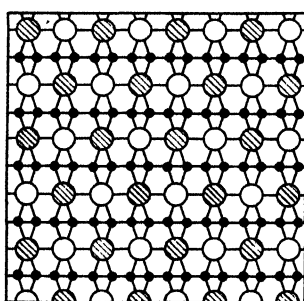
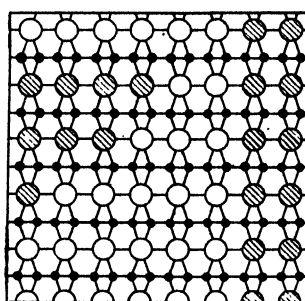



Fig. 11 Fig. 12 Fig. 13

Homogeneous states of the compound AB_2. ○ A, ● B, ◍ E.
Fig. 8. Solid – Fig. 9. Liquid – Fig. 10 Gaseous. – Fig. 11 Homogeneous solution of EB_2AB_2 in.

Mixed crystals $(A,E)\ B_2$.
Fig. 12. Homogeneous – Fig. 13 Heterogeneous.

To answer this question we must deal shortly with the phase theory, which treats of relations between homogeneous states.

c. *Conception of Phase*

According to the thermodynamical definition *any homogeneous state is called a phase*. Fig. 8–12 thus picture the structure of phases, while in Fig. 13 no homogeneous phase but a heterogeneous system of AB_2 and EB_2 is given.

Originally it was believed that the colloid particles were homogeneous and therefore the dispersed particles were characterized as *dispersed phase* and the surrounding liquid as *dispersing medium* (Fig. 14). A sol represents therefore a two-phase system. Now, naturally, the study of the structure of colloids need not be confined to the liquid state. In the microscopical domain dispersions of liquid or solid particles in liquid or solid media are known (emulsions, suspensions, etc.). We may also expect them in the submicroscopic domain. Since, however, the particles in such dispersions are no longer visible, they were designated as *dispersoids*. In this way one tried to characterize not only the structure of sols but in the most general sense the structure of all colloids, as will be clear from the following system (Wo. Ostwald, 1909).

System of dispersoids. According to the theory of dispersions each of the three states of matter: solid, liquid or gas can occur either as dispersing medium or as

dispersed phase (Fig. 14), so that $3^2 = 9$ combinations are possible (Table II). Fig. 14 shows how in these systems the dispersed phase I is distributed in the dispersing phase II.

TABLE II

DISPERSOID SYSTEMS, ACCORDING TO WO. OSTWALD, 1909

Dispersing medium	Dispersed phase	Dispersoids
Solid	Solid	Grain-structure
Solid	Liquid	Drop-structure
Solid	Gas	Bubble-structure
Liquid	Solid	Suspensoids
Liquid	Liquid	Emulsoids
Liquid	Gas	Foams
Gas	Solid	Smoke
Gas	Liquid	Mist
Gas	Gas	—

On the strength of the definition of phases it was originally believed that the dispersed part I was homogeneous. In the dispersoids, however, this leads to difficulties. Often it was doubtful whether a phase was liquid or solid. For, suppose the dispersoid particles to become smaller and smaller until they contain only a small

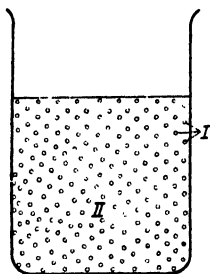
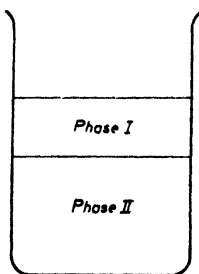
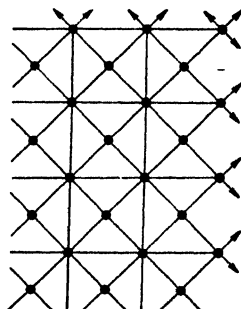



Fig. 14 Fig. 15 Fig. 16

Fig. 14. Colloid chemical concept of phase. I Dispersed phase, II dispersing medium. The inhomogeneity of boundaries reigns throughout the system – Fig. 15. Thermodynamical concept of phase. The homogeneity of the phases reigns throughout the system – Fig. 16. Inhomogeneous surroundings of the lattice points of boundary planes (face-centered cubic lattice).

number of molecules, then it would be difficult to decide whether they are solid or liquid. Liquid drops can be considered as homogeneous, while it is very difficult to prove this of solid suspended particles. It was only by the introduction of X-ray methods in colloid chemistry (that is, relatively late) that it has been possible to prove that the particles of certain dispersoids, for instance gold and silver sols, possess a crystal lattice and are therefore really homogeneous. With increasing degree of dispersion, however, the homogeneity of a crystal lattice also becomes questionable. For, the energy of the points lying in its surface is different from that of the points inside the lattice, because they are no longer surrounded on all sides by equivalent fields of force (Fig. 16). In the case of liquids this gives rise to the surface tension.

For instance, in the smallest gold particles which can be measured by X-ray methods (SCHERRER, 1920), 200 of the 380 Au-atoms, i.e., more than half the total number, lie in the surface of the crystals, and with decreasing particle size these numbers compare, of course, still less favourably, until with 14 or still less atoms all lie in the surface (face-centred cube, Fig. 25). Thus one can no longer speak of a homogeneous phase in the case of atoms that are not similarly surrounded on all sides. It is only by a still further increase in dispersion that finally a homogeneous molecularly dispersed, solution of Au-ions is obtained.

On the other hand it has been ascertained (ZSIGMONDY, 1925, p. 39) that the homogeneous primary particles of suspensoids can cluster together to form bigger heterogeneous secondary particles (compare Fig. 75) without any fundamental change in the properties of such sols. This strengthened the opinion that the properties of sols and other colloids were not decided by the inner structure of the particles. Since with increasing dispersion the surface of the particles increases considerably in proportion to their mass, colloid chemistry has developed much more into a science of surfaces. The properties and reactions of colloids have been elucidated to a great extent by the study of surface reactions. Whereas the phase theory is concerned with the equilibrium between different phases and is able to predict under what conditions phases cease to exist (dissolution) or new phases appear (separation into two layers), classical colloid chemistry is interested in the first place in *phase boundaries* (capillary chemistry according to FREUNDLICH, 1922).

Thermodynamics require that all parts of a phase have exactly the same energy content. This is only realized, however, when the phases are so extended that the irregular distribution of energy at their surface, i.e., the inhomogeneity in the immediate neighbourhood of the phase boundary (Fig. 16) can be neglected (Fig. 15). Thus, the classical phase theory has to forego all considerations concerning phase boundaries (compare Fig. 3–11) because of their inhomogeneity, and its laws only apply to homogeneous regions of at least microscopic dimensions. On the contrary, the properties of colloids are determined in the first place by the inhomogeneity of the phase boundaries whose effect is so predominating as a result of the very large surface. For this reason it has been suggested by OSTWALD (1938) that the definition "dispersed phased" should be avoided, and that we should speak of the "colloid portion" of the dispersoid.

Originally the phase theory seemed appropriate to the purpose of explaining the formation of new phases (separation into two strata, formation of vacuoles) or the disappearance of phases (melting-in) in biological systems. From the above, however, it is clear that the phase theory does not hold good in colloid chemistry, since it has been developed by emphasizing the homogeneity of the phase and neglecting the specific properties of surfaces, while conversely, in cytologic systems, homogeneity usually fails and the surfaces are of quite extraordinary importance. BUNGENBERG DE JONG and his fellow-workers have elucidated the principles according to which visible boundary layers can appear and disappear in those heterogeneous systems to which the phase theory does not apply. In his theory of coacervation BUNGENBERG DE JONG has summarized the rules which govern these phenomena.

d. *Coacervation*

In the separation of a sol into two non-miscible parts the dispersing medium and the dispersed portion often do not separate completely. Flakes are formed which

still contain a certain amount of dispersing medium and therefore remain suspended. For this reason the flocculation is usually reversible. If however such flakes collect into small drops or into a coherent liquid layer, we have to do with a phenomenon, for which BUNGENBERG DE JONG introduced the term *coacervation* (Fig. 20); in English: piling up (acervus = pile).

Hydration. The colloid particles in a sol are solvated, which means that molecules of the dispersing medium adhere to the particle. In the special case of water, this solvation is designated as *hydration*, since in that case water molecules are bound by the colloid particle. The attraction is brought about by electrostatic forces, for, in a water molecule the electric charges are not distributed uniformly, because the

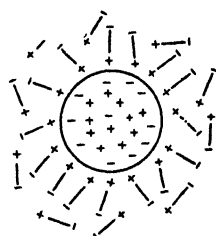
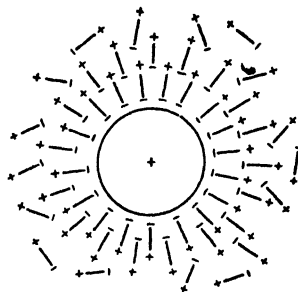



Fig. 17 Fig. 18 Fig. 19

Fig. 17. Model of a water molecule and scheme of dipoles – Fig. 18. Hydration of an iso-electric colloid particle – Fig. 19. Hydration of a charged colloid particle (according to PALLMANN, 1931).

two positive hydrogen atoms are separated in space from the doubly charged negative oxygen. For that reason a water molecule in an electric field behaves like a molecular rod with two different electric poles and is therefore designated as a *dipole* (Fig. 17). Similarly, in a colloid particle the electric charges are usually not distributed uniformly, not even if the particles are iso-electric, i.e., if their positive and negative charges cancel each other so that outwardly they appear neutral. In Fig. 18 a particle has been sketched, the negative charges of which are situated towards its surface. This has a polarizing effect on the water molecules in the immediate neighbourhood of the particle. These water molecules follow the particle in its Brownian movement as the so-called solvation or hydration layer. If the colloid particle is not neutral but carries an excess negative or positive charge as a result of dissociation of H- or OH-ions, the swarm of oriented dipoles surrounding the particle will be correspondingly larger. This is why the hydration of colloids reaches its minimal value at the iso-electric point.

The binding forces which attract the water dipoles decrease with increasing distance. Thus the swarm of water molecules which are hampered in their free movement becomes less dense in the outer layers until in the end one reaches without noticeable transition the region of the freely moving dipoles of the dispersing medium. In the solvation layer the density of the water therefore decreases exponentially, in much the same way as the density of the atmosphere, with increasing distance from the earth. Since no sudden transition exists from the hydration layer to the free water,

such hydrophilic colloids are very stable. The particles show no tendency to cluster together; in a way they "have no surface at all", their surface energy is zero (Fig. 20a).

Dehydration. If water is withdrawn from the diffuse solvation layer, the difference between bound and freely moving dipoles becomes noticeable. The water layer around the particle now acquires a surface (Fig. 20b) and if two such dehydrated particles meet, the surface energy which tends to a minimum value will cause the surrounding water layers to unite. The colloid particles, however, cannot come into touch with each other because of their solvation layers. However, they no longer possess separate layers, for these have all united into a single liquid sphere. If the number of particles united in this way becomes so large that they form a microscopically visible conglo-

Fig. 20. Dehydration of colloid particles (according to BUNGENBERG DE JONG, 1932). *a*) Diffuse hydration layer, *b*) definite hydration layer, *c*) beginning coacervation.

meration, one speaks of flakes or flocculates. These can further cluster into drops (microcoacervation) and finally into a liquid layer (macrocoacervation). Thus coacervates are liquids rich in colloid which have been separated by means of dehydration.

In the coacervate the distribution of colloid particles is statistically uniform, as in the original sol, although the density has been increased. If the colloid particles are considered as dispersed phase, their state has not been changed in the coacervation process. And yet clearly a new phase boundary is formed between a layer rich in colloid and one poor in colloid. This example shows the vagueness of the concept phase in colloid chemistry. For that reason the hydrophilic sols and the coacervates originating from them are sometimes designated as quasi-homogeneous phases, since the distribution of the particles is completely uniform and the particle size can sometimes decrease to molecular dimensions.

The dehydration of colloid particles illustrated in Fig. 20 can be achieved in various ways; for instance, a rise in temperature, which accentuates the contrast between bound and freely moving water will often suffice. Usually, however, one makes use of dehydrating substances such as salts (salting out) or aliphatic alcohols or acetone. Such substances which disturb the stability of the sol and further the tendency to demix are called *sensibilisators*. Besides salts and organic liquids colloidal solutions may also be used as sensibilisators if they compete with the particles of the original sol to bind the free water and in such a way cause dehydration.

The dispersing medium which is separated from the coacervate is designated as *equilibrium liquid* (Fig. 21), for, according as changes in temperature or composition take place in the system, water is taken up or given off by the coacervate. The situation is, therefore, analogous to that occuring in the demixing of phenol and water (p. 33). Coacervates can be regarded as a solution of water in the colloid (swelling) and the equilibrium liquid must then represent a solution of a small amount of colloid in

water. In the example given in Fig. 21, however, the gelatine is insoluble in water and the concentration of the colloid in the equilibrium liquid is practically zero. Here the analogy can, therefore, not be carried further, since instead of a reciprocal solubility there exists only a one-sided assimilation of water by the colloid. The reason why gelatin is completely insoluble below its melting point will be made clear on p. 53. Coacervates of homopolar substances have been studied by Mme DOBRY (1938, 1939).

Discharge. In biological systems the colloid particles are seldom neutral; usually they are electrically charged. Particles carrying opposite charges tend to unite, but

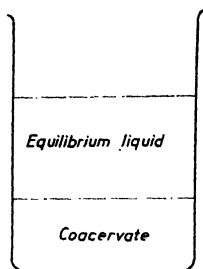



Fig. 21. Coacervation of gelatin at 41° C. Isoelectric gelatin sol + alcohol as sensibilisator. Equilibrium liquid = solution of water and alcohol. Coacervate = gelatin + small amounts of water + alcohol.

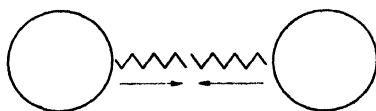



Fig. 22. State of stability of colloid particles (according to BUNGENBERG DE JONG and BONNER, 1935). Attraction by opposite electrical charges (arrows). Repulsion by solvation layer (spring).

because of their solvation layers can only approach each other to a certain extent. The attraction is counteracted by the hydration as by a spring (Fig. 22) and thus no coagulation takes place which would annul the charges, but again a coacervate occurs which now contains particles of opposite charge. So, in addition to sensibilisators electric charges can cause coacervation. To this end the sols must have opposite charges, for instance: gelatin (positive) and gum arabic (negative) or lecithin (positive) and nucleic acid (negative). In this case the aggregation is designated as *complex coacervation*, since two oppositely charged kinds of particles take part in the flocculation. In many cases colloid particles can be made to revert their charge by adding neutral salts. In this case the well-known valency rules apply: on the addition of polyvalent cations, negative particles change their sign more easily according as the valency of the cation is higher, while positive particles behave in a similar way with respect to polyvalent anions. Negatively charged phosphatides, for example, reverse their charge on the addition of $CaCl_2$. In the sol the phosphatide particles which have already become positive and those which as yet remained negative attract each other, and in this way a demixing occurs which has been called *autocomplex coacervation*, because in this case similar but oppositely charged particles attract each other.

Morphologically the coacervation shows a great many phenomena which have their counterpart in the phenomena occurring in cells. In the first place the *vacuolisation* should be mentioned. If in a system consisting of equilibrium liquid and suspended coacervate droplets the equilibrium is changed as a result of changes in temperature or composition in the direction of a further dehydration (heating, further addition of sensibilisators), vacuoles appear in the droplets. These represent separated equilibrium liquid which has remained inside the coacervate droplets (Fig. 23). It is probable that vacuolisation by dehydration is comparable with the formation of vacuoles in the

cell, since, in that case too, liquid is being separated from the plasma colloids.

Apart from this striking analogy BUNGENBERG DE JONG (1932) mentions still further models for cytological differentiation on the basis of observations with coacervates. When mixing sols of gelatin, gum arabic and nucleic acid from yeast, in addition to equilibrium liquid two complex coacervates arise, one of which consists mainly of gelatin and gum arabic, while the other is composed chiefly of gelatin and nucleic acid. Their partition is such that the first always contains the second in the form of enclosed droplets.

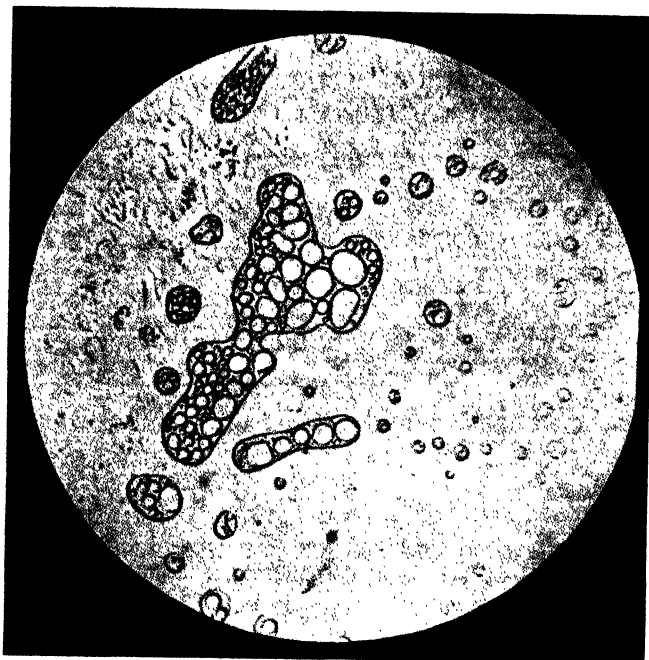



Fig. 23. Vacuolisation by lowering the temperature of coacervate drops consisting of gelatin sol + resorcine (from BUNGENBERG DE JONG, 1932).

This can easily be demonstrated, since the negative nucleic acid coacervate can be selectively dyed by alkaline dyes such as methyl green. This is regarded by BUNGENBERG DE JONG as a model for a nucleus imbedded in cytoplasm. Personally, however, I do not believe that such comparisons are admissible since both nucleus and cytoplasm possess a structure, whereas the liquid coacervate droplets are completely amorphous. For that reason, the picture suggesting the resemblance to the cell may be rather incidental and therefore should not be used in analogy to cytological phenomena. There would otherwise be too great a temptation to oversimplify the relationships between cytoplasm and nucleus. The nuclear changes in karyokinesis for instance cannot possibly be attributed to changes in hydration or electric charges alone. These phenomena are attended by complicated structural changes.

Whereas in the beginning the theory of coacervation was principally concerned with the surfaces of the colloid particles with their solvation layers and electric charges, and was an attempt to gain more knowledge about the structure of boundary

layers (see p. 30 and 169), its interest has recently been directed also towards the inner structure of coacervate systems. In biological objects we must attribute a submicroscopic gel structure to the coacervate (BANK, 1941). Therefore, apart from a knowledge of boundary structure, we are also in need of a deeper insight into the inner structure of colloid particles and coacervate flocculates. In order to advance in this direction we must resort to structural principles.

§ 2. PRINCIPLES OF STRUCTURE

By structural principles we mean the laws governing the mutual position of atoms, ions, and molecules. The positions of the atoms in the molecule are studied by *structural chemistry*, which in this respect appears as a morphological science. For example, when we represent the carbon atom by its 4 valencies or a benzene ring by the well-known hexagon (Fig. 24), these are morphological illustrations based on certain properties of these substances. The exact location of the valency bonds in space and the distances between the atoms remained unknown for a long time, and a certain arbitrariness was in force at the use of valency lines as regards their direction and length (compare Fig. 35b). To-day, however, the data needed for an exact morphological representation are known, and, if written in a suitable way, at least the simpler chemical formulae actually do represent molecular models, which have been projected on to a plane. We owe our knowledge of the exact distances and directions chiefly to X-ray analysis. X-rays allow of measuring dimensions of the order of magnitude of their wave-length (e.g., copper radiation: $\lambda = 1.54$ Å), if identical distances are often repeated and act as a lattice, causing interferences which can be photographed and thus made macroscopically visible. It is, therefore, the principle of *repetition*, which has enabled one to penetrate into the morphology of molecular structure. The more regularly the given distances are arranged, the more accurately the absolute values and directions can be determined. From the considerations concerning homogeneity it follows, therefore, that in gases, liquids (Fig. 9–11) and solutions the morphology of the molecules cannot be determined by means of X-rays. An exception, it is true, is made by solutions of very large molecules which in their own construction show a certain periodicity (for example carbon chains). In such cases however, the measurements are often not unambiguous, because the molecules are not orientated in fixed directions. The most reliable values of atomic distances, which often reach an almost unbelievable precision (up to $1^0/_{00}$ of 1 Å) have therefore been determined in crystal lattices. For a quantitative determination of the arrangement of the atoms in a molecule necessarily one must make use of phases which possess a *structure*. Amorphous phases without structure, such as liquids and real solutions are not suited for the elucidation of such morphological relations.

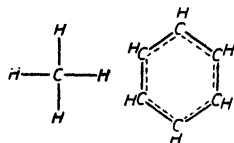



Fig. 24

Now, in biology the circumstances in this respect are especially unfavourable. Although we must attribute a structure to the protoplasm, this is not governed by the principle of repetition to such an extent that X-ray analysis would be possible. It is true that periodicity plays an important rôle in all living matter, but more with respect to time than to the arrangement in space. A strictly periodic order presupposes an equilibrium of forces and this contradicts life, which rests on movement and on the

maintenance of non-equilibria. As soon, however, as a chemical substance is withdrawn from the metabolic process, the ordering forces can intervene and form periodic structures, as for example with the skeleton substances cellulose, chitine, collagene, keratin, etc. Therefore, to study the structure of protoplasm other methods should be applied which, however, are partly based on the results of the investigations on crystal structure. For this reason this important branch of morphology must be briefly touched upon.

a. *Crystal Structure*

Lattice. The essential nature of lattices is determined by the fact that certain locations of points, which in the more simple cases are identical with the centre of gravity of the atoms, periodically repeat themselves in three given directions in space. These directions coincide with the axes of the crystallographic system. The distance from one point to the next, identical one is designated as identity period or spacing. Depending on the crystallographic system, the spacings are the same in either three (cubic) or only two directions (tetragonal, hexagonal, rhomboedric), or they are different in all three dimensions (rhombic, monoclinic, triclinic). The regularly repeated points form an *array* of points. Displacing such a row by constant amounts in a direction either perpendicular or oblique to its own direction, we obtain the *lattice plane*, while finally the *crystal lattice* results from displacing such a plane. If a point in the lattice is moved in the three principal directions, each time covering the identity period concerned, and if the three vectors obtained are completed to a three-dimensional parallelepiped, we obtain the so-called *base* or *unit cell* of the crystal lattice. In analogy with a gas molecule which represents the smallest unit with all the *chemical* properties of the gaseous phase the elementary cell is the smallest unit which still shows all *physical* and symmetry properties of the crystal. It can contain one or several molecules (and in the case of high polymers even parts of molecules). We have, therefore, to deal with a *geometrical* concept and by no means with a chemical one. If the unit cell is decomposed into its elements, the crystalline properties are lost. As the base cell possesses all the properties of the crystal, and this crystal can be obtained by displacing the elementary unit in the principal directions, structure analysis aims at determining the dimensions and the symmetry of the base. Its shape is determined by three identity periods a : b : c in Ångström units, to which in monoclinic and triclinic systems one must add the angle β, resp. the angles α, β, γ formed by the sides of the unit cell. The macroscopically determined proportions between the axes of the crystals agree with the proportions between the dimensions of the unit cell, provided analogous planes are considered.

X-ray analysis measures the distances between the lattice planes. In the case of crystals showing a high degree of symmetry (cubic system) the lattice points are identical with the points of intersection of symmetry planes and their distances can therefore be calculated from the distances in the X-ray diagram. In the case of lattices having a lower degree of symmetry, however, the situation of the points in the lattice planes is not determined unambiguously by symmetry elements; they possess certain degrees of freedom. Accordingly, the determination of the structure with the aid of the distances in the X-ray diagram alone is not possible; one must also resort to measurements of the intensity of the interferences. In which case, however, often only an approximate determination of the exact situation of all lattice points in the unit cell is possible.

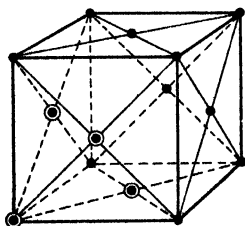



Fig. 25

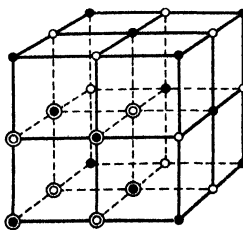



Fig. 26

Crystal lattices. The surrounded points belong to the unit cell.
Fig. 25. Gold. $a = 4.07$ Å, ● Au. – Fig. 26. Sodium chloride. $a = 5.60$ Å, ● Na, o Cl.

In Fig. 25 and 26 two of the best-known crystal lattices have been drawn, to wit that of the element gold and that of the compound sodium chloride. Both lattices are cubic; this means that the dimensions and the shape of the unit cell are determined by a single identity period *a* which is the same in three mutually perpendicular directions. Once the spacing *a* has been determined by means of X-rays, the volume a^3 and, from the known density of the crystalline substance, the weight of the unit cell can be calculated. Dividing this weight by the absolute weight of the atom or molecule concerned (= atomic or, as the case may be, molecular weight/LOSCHMIDT's number $0.606 \cdot 10^{24}$), one finds the number of atoms or molecules in the unit cell.

For example, the elementary cell of gold contains 4 Au-atoms, that of sodium chloride 4 Na- and 4 Cl-ions. These points have been encircled in Fig. 25 and 26, the other points marked on the planes of the cube must be considered as having originated from the encircled ones by a simple translation, and thus belong to a neighbouring unit cell. The lattice type of gold is designated as face-centred because the points of intersection of the diagonals of the faces are all occupied by atoms. According to the same scheme, although with different identity periods *a*, numerous elements such as Ag, Cu, Al, Pb, etc. crystallise. In the NaCl type lattice, which is found in several binary compounds (NaF, KCl, PbS, etc.) with different values of *a*, two of such face-centred cubic lattices overlap.

Primary valence lattice. Next to the geometrical relations between the points in the crystal lattices, the forces which keep the atoms together are of primary importance. The purely geometrical consideration of the lattice is quite independent of this. As soon, however, as one is interested in the reason why certain distances in a lattice are great and others small, this question must be considered. Now, the lattice forces are in fact of various nature. Indeed in the examples mentioned the forces are different. In Fig. 25 similar atoms, in Fig. 26 oppositely charged ions attract each other. In both cases *primary* valencies act as lattice forces which can join together similarly charged as well as oppositely charged particles. In the first case one speaks of a *homopolar lattice*, in the second of a *heteropolar* or *ion lattice.*

The morphological similarity of these two types of lattice is due to the fact that in both cases the construction of the lattice is founded on the rules of the theory of *coordination.* According to WERNER's chemistry of complexes, each atom is surrounded by a fixed number of neighbouring particles, either 4, 6, 8 or 12, dependent on volume conditions. This theory, based originally on the composition of salts containing crystal water [e.g., $Ca(H_2O)_6Cl_2$] and other complex salts, has also proved useful in the elucidation of crystal structures of other compounds and of the elements. In fact, in Fig. 25 each Au-atom at the corners of the cube is surrounded by 12 neighbouring atoms and in Fig. 26 each Na-ion by 6 Cl-ions or, vice versa, each Cl-ion by 6 Na-ions.

The theory of coördination has led to another fundamental recognition which has become of the greatest importance to the submicroscopical morphology of organic compounds. It could be shown that the lattice points in Fig. 25 and 26 represent only the centres of gravity of the atoms. The range of their electron orbits, however, extends over such large volumes that these can be represented by spheres touching each other in the lattice (Fig. 27–29). A crystal lattice, therefore, which is kept together by main valencies is much more closely packed than the common pictures suggest. Unfortunately one does not get very elucidating representations in space if one draws continuous spheres instead of lattice points, whereas in a plane this procedure can be applied with great success (compare Fig. 31). The atomic distances in the lattices of elements correspond, therefore, to the atomic diameters and in binary compounds they represent the sum of the radii of the two partners (GOLDSCHMIDT). In this way it has been possible to determine the volume occupied by various atoms and at the same time to find an explanation for the different coordination numbers. For example,

four Cl-atoms combined in a tetrahedron together enclose a space which just answers the size of a silicon atom; this accounts for the coordination number 4 in the compound $SiCl_4$. Of the smaller fluorine atoms, however, we need 6 spheres to obtain the space occupied by one Si-atom. Hence the coordination number 6 (SiF_6).

If the lattice contains homopolar valence bonds, the distances between the atoms, respectively the diameters of their spheres show a surprising constancy, not only in simple compounds, but also in very complicated ones. In the heteropolar ion lattices a disturbing effect occurs because of the opposite charges of the two partners. The

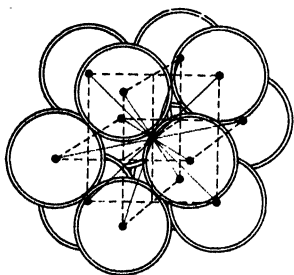
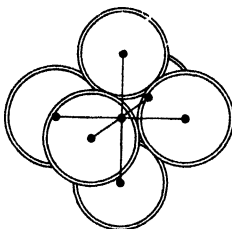
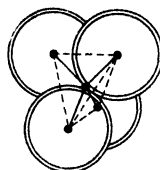



Fig. 27 Fig. 28 Fig. 29

Coordination numbers (according to MAGNUS, 1922)

Fig. 27. Number 12; e.g., Au $(Au)_{12}$ in crystallised gold. Fig. 28. Number 6; e.g., $Na(Cl)_6$ in sodium chloride; $Fe(CN)_6$ as ion. Fig. 29. Number 4; e.g., CCl_4, $C(C)_4$ in diamond.

ions have a polarising effect upon each other, which can lead to *deformations* of the electron orbits (FAJANS) in those cases where the symmetry of the lattice does not exclude such effects, as for instance in the lattice of NaCl (Fig. 26). The ions can then no longer be represented by spheres, they represent dipoles similar to the water molecules in Fig. 17. The result is, that ion lattices often possess a low symmetry and that the atomic distances between given partners are subject to certain fluctuations, dependent on the special circumstances.

Fortunately this does not apply to the structures of organic compounds which always have a homopolar character; the distances found in certain compounds can therefore be transferred with great certainty to other ones, so that one can speak of *distance rules*. In Table III a number of atomic distances are given as determined in organic crystals by means of X-rays. In these considerations the hydrogen atoms must be neglected, since they do not scatter X-rays; nor do they seem to have a perceptible influence on the distances between the atoms. Table III, for example, shows that in single bonds the atom radius of carbon r_C amounts to 0.77 Å and that of nitrogen, r_N, to 0.71 Å. In spite of the larger atomic weight of nitrogen its sphere of action is smaller than that of carbon. One observes further that the sphere of influence of the carbon atoms is decreased by double bonds.

Each valency in an organic molecule corresponds to a definite amount of energy (MEYER and MARK, 1930). In the combustion of the homologous paraffins, for instance, the heat of combustion per mole increases by a definite amount for each new C-atom introduced; this value amounts to about 70 kcal. The energy equivalents for the other compounds mentioned in Table III have been determined in a similar way. It will be apparent that with decreasing distance between the C-atoms the energy content of the different bonds increases.

TABLE III

DISTANCES AND MAIN VALENCY FORCES BETWEEN THE ATOMS IN ORGANIC COMPOUNDS

Crystal lattice	Bond	Distance in Å accord. to STUART, 1934	Energy-equivalent kcal (MEYER-MARK, 1930)
Diamond	Aliphatic C—C	1.54	71
Graphite	Aromatic C⩵C	1.42–145	96
Stilbene	Double C=C	1.35	125
Ca-carbide	Triple C≡C	1.19	166
Carbonic acid	Ketone C=O	1.05–1.15	203
Polyoxymethylene	Oxygen bridge C—O	1.49	—
Urea; hexamethylene tetramine.	Amino C—N	1.33–1.48	—

To sum up, it can be said that in the main valence bonds which play a part in the structure of protoplasm, distances of 1–1.5 Å and bond energies of the order of 100–200 kgcal occur.

Molecule lattice. In addition to homopolar main valence lattices and heteropolar ion lattices we must consider molecule lattices. If the valency of an atom species corresponds to the coordination number with regard to another atom species (as, for instance, in CH_4), the mutual saturation of the valencies excludes the possibility of unlimited lattices such as those shown in Fig. 25 and 26. Although such molecules no longer possess free valencies, they can still be arranged in a crystal lattice (see MARK and SCHOSSBERGER, 1937). The binding forces, however, are now of a different nature; in contrast to the primary valencies they are called *secondary valencies.* They are explained in theoretical physics by means of dipole moments, in much the same way as the orientation and attraction of water molecules by an ion (see Fig. 19). In practice these forces between the molecules cause the *cohesion.* The secondary valence forces are, therefore, identical with the VAN DER WAALS cohesive forces. In molecule lattices they are of the same nature as in liquids and they can therefore be derived from the heat of sublimation or vaporisation of the compound. It then becomes apparent that each atom or radical occurring in the structural formulae of organic chemistry contributes a certain amount to the cohesion. To a first approximation the cohesion of a molecule species is composed additively of these partial contributions, and can be calculated by adding up the various increments, in exactly the same way as the molecular volume (according to KOPP's rule), the molecular weight or the molecular refraction. Accordingly, the contribution of the characteristic groups to the cohesion has been denoted by *molar cohesion* (MEYER and MARK, 1930). For example, the heat of vaporisation of ethyl alcohol which amounts to 10 kcal per mole is additively composed of the molar cohesions of CH_3, CH_2, and OH. The values concerned can be found in Table IV.

This table shows that in neighbouring molecules methyl and methylene groups, and also oxygen bridges attract each other only slightly. The attraction between amino and ketone groups is twice as large, and in the polar hydroxyl and carboxyl groups the cohesion assumes quite considerable values. None the less, all the values for molar cohesion are 10 to 100 times smaller than the energy equivalents of the main valence bonds, and accordingly the secondary valence bonds are at least 10 times

TABLE IV

COHESIVE FORCES BETWEEN ORGANIC GROUPS, ACCORDING TO MEYER AND MARK 1930

Groups		Molar cohesion kcal/mole
Aliphatic C: methyl and methylene groups	$—CH_3$ and $=CH_2$	1.78
	$—CH_2—$, $=CH—$	0.99
Ether bridge	—O—	1.63
Amino group	$—NH_2$	3.53
Carbonyl group	=CO	4.27
Aldehyde group	—CHO	4.70
Hydroxyl group	—OH	7.25
Carboxyl group	—COOH	8.97

weaker. Consequently, whenever secondary valencies play a decisive rôle in the crystal lattice, the distances are much greater than those between atoms bound by primary valencies. In organic crystals, therefore, in which both bond types occur: primary valencies inside the molecule (intramolecular) and secondary valencies between the molecules (intermolecular), the lattice distances are essentially of two different orders of magnitude.

b. *Structural Chemistry*

After the discovery of *stereoisomery*, structural chemistry learnt to distinguish between different positions of the substituents to the carbon atom. At first, the results of this interesting science (WERNER, 1904; FREUDENBERG, 1933) were of a rather qualitative nature, and referred mainly to the directions radiating from the C-atom. Quantitative determinations of distances along these directions were not yet possible. The results of crystal structure, however, determine not only qualitatively but also *quantitatively* the *relative positions* of the atoms in space.

The starting point for the new development in structural chemistry was the crystal lattice of diamond, which crystallises in the cubic system. Its unit cell is a cube containing 8 C-atoms, 4 of which belong to a face-centred cube as in the case of gold, while the four remaining atoms are situated on the body diagonals half-way between the corners of the cube and its centre (Fig. 30a). Thus the unit cell contains, as it were, 4 central atoms surrounded by 4 neighbouring atoms at the corners of a tetrahedron, in conformity with their coordination number (Fig. 29). If this three-dimensional lattice is projected on to its base, Fig. 30b is obtained, which shows the arrangement of valency lines commonly used in organic chemistry! Thus

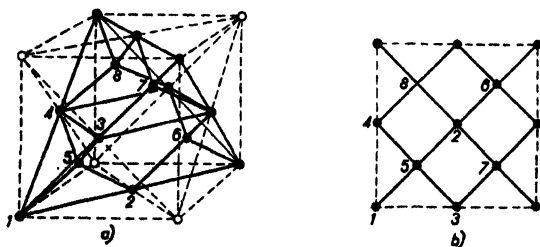



Fig. 30. Diamond lattice. *a*) Unit cell, *b*) projection.

the usual scheme of the fourvalent carbon (Fig. 24) is morphologically correct if it is considered as the projection of a tetrahedron.

According to X-ray analysis, the lattice period of diamond, i.e., the edge of the cube, measures 3.55 Å. It follows that the distance between the lattice points on the face diagonal is $\frac{1}{2} \cdot 3.55 \cdot \sqrt{2} = 2.51$ Å; the shortest distance between two C-atoms on the body diagonal is $\frac{1}{4} \cdot 3.55 \cdot \sqrt{3} = 1.54$ Å. It is in this simple way that the C—C-distance corresponding to the sphere of action of a C-atom in an aliphatic bond has been calculated (Table III).

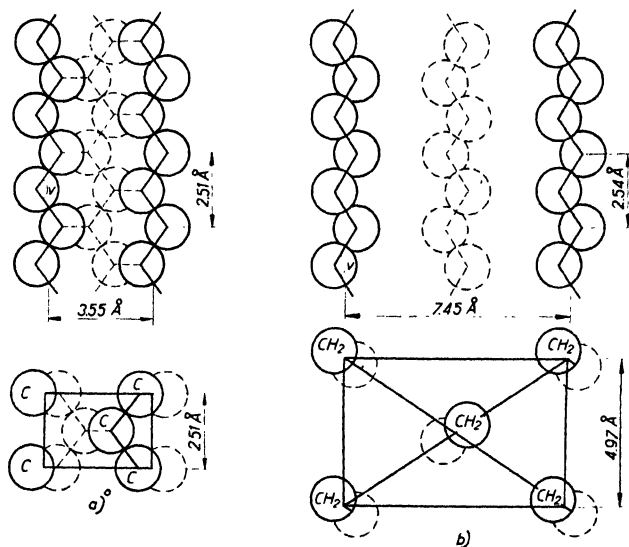



Fig. 31. *a*) Diamond lattice (primary valency lattice) as compared with Fig. 30a by 45° inclined. *b*) Paraffine lattice (molecule lattice); v = valency angle = 109°.5.

If a plane is drawn through two body diagonals the arrangement of lattice points obtained is represented in Fig. 31. In this cross-section the C-atoms are joined by a zig-zag line whose links enclose the so-called tetrahedron angle of 109°.5. On parallel planes further arrays of such zig-zag chains are found, one of which has been represented in Fig. 31a (dotted curve). It is linked up with the other two by primary valencies.

Aliphatic compounds (*chain lattice*). The zig-zag ordering described is fundamental to the morphology of saturated carbon compounds; for it has been found that all aliphatic molecules represent such kinked chains. In paraffin molecules, for instance, the increase in chain-length for each additional C-atom is 1.27 Å instead of 1.54 Å. It can easily be calculated that this is in conformity with the zig-zag chains showing the tetrahedron angle. In this way two carbon atoms reach a spacing of 2.54 Å, which is the intramolecular period of the paraffins (HENGSTENBERG, 1928; MÜLLER, 1929; HALLE, 1931).

In Fig. 31b it is shown how by parallel alignment such chains combine into the rhombic crystal lattice of the paraffins. It seems paradoxical that the soft, plastic paraffin crystals should have a lattice structure so similar to the diamond model represented by Fig. 31a. Notwithstanding the apparent analogy, however, there exist fundamental differences which explain the differences in the physical behaviour of the two substances. In particular the lattice of the paraffin crystals is built much more loosely. This is caused by the fact that these crystals do not possess a main valency

lattice but a molecule lattice. The chains are joined by VAN DER WAALS forces only, since the CH_2 groups are able to bind only two neighbouring groups by primary valencies. Thus in the paraffin lattice we have two orders of distances: molecular distances of the order of magnitude 5 Å and atomic ones of the order of magnitude 1.5 Å (Fig. 31b). The fact that in the diamond lattice all C-atoms touch each other explains its great density and hardness. The paraffin lattice, on the contrary, causes

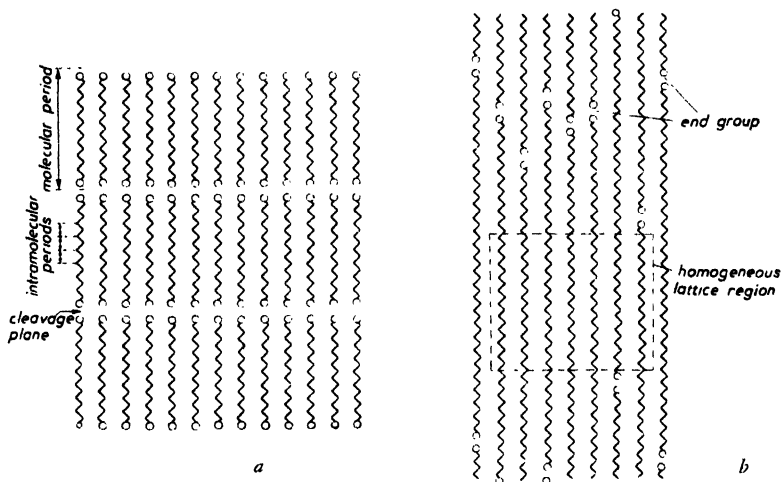



Fig. 32. Aliphatic chains. *a*) Molecule lattice, *b*) chain lattice.

a much smaller density, and layers of molecules can relatively easily be shifted with respect to each other (Fig. 32), which accounts for the softness and plasticity of paraffin crystals.

As long as the paraffin chains are short, they easily crystallise into a molecule lattice. This leads to crystals in the form of flakes, which are cleavable along the base (Fig. 32a). When, however, the chains become very long, it becomes increasingly difficult to arrange the terminal groups in fixed planes, and crystallisation takes place as pictured in Fig. 32b. Here no rigorous lattice order prevails, since a length-ways displacement of the chains with respect to each other, over distances equal to an intramolecular spacing, i.e., only a fraction of the chain-length, does not affect the lattice structure. This is due to the fact that the smaller spacings inside the molecule (2.54 Å in the case of paraffins) become so important on account of their large number, that the periodicity of the endgroups loses its significance. Such arrangements of long molecules are denoted as *chain lattice*. It is to be noted that the chains cannot revolve around their longitudinal axis, since otherwise there would be no lattice order. The cross-section of the chain lattice is, therefore, homogeneous. Lengthwise, however, one meets inhomogeneities which in Fig. 32b are indicated by marking the endgroups. When the chains are very long, homogeneous lattice regions can evidently also be cut out length-wise.

Aromatic compounds (*layer lattice*). Unlike the aliphatic compounds, the aromatic ones cannot be derived from the structure of diamond. Their structure is similar to that of *graphite*. This modification of carbon crystallises in the hexagonal system and possesses a crystal lattice as represented in Fig. 33. The carbon atoms form rings containing 6 atoms, which are linked together in an uninter-

rupted plane. Thus at each lattice point 3 primary valencies are engaged. The fourth valency is distributed among the neighbouring atoms as in the benzene ring (Fig. 24). Accordingly, as a result of the larger bond energy, the C—C-distance is reduced to 1.45 Å (see Fig. 33). As all primary valencies are thus engaged in a plane, the resulting main valency layers are united in a lattice by weaker secondary valencies. The distance between the layers (3.41 Å) is therefore considerably larger than that in the rings. A structure in which the lattice forces and spacings within a plane are so strongly different from those in a direction perpendicular (or nearly perpendicular) to this plane is called a *layer lattice.* Compounds of this lattice type always crystallise in the form of flakes and are usually easily cleavable along the base (mica, serisite). Many benzene derivatives and other aromatic compounds (naphtalene, anthracene, etc.) belong to this class. The division into aliphatic and aromatic substances is therefore not only based upon their chemical behaviour, but it also has a morphological background in that the one tends to crystallise into a chain lattice, while the other shows a strong tendency towards the development of a layer lattice.

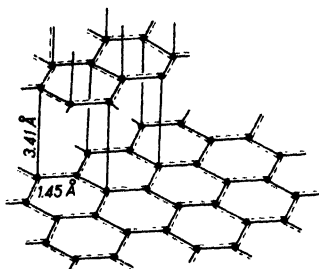
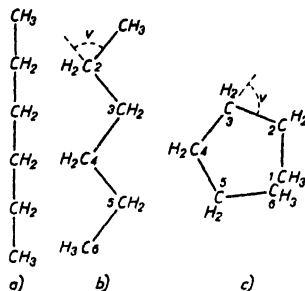



Fig. 33 Fig. 34

Fig. 33. Graphite lattice. – Fig. 34. Hexane. *a*) Conventional structural formula; *b*) morphologically correct formula; *c*) ring constellation, supplement to the valency angle v = 70°.5.

Cyclic compounds. The structural formulae of aliphatic chemistry show a great similarity to molecular models if in drawing them one takes into account the valency angle between two successive C-C bonds. A chain such as hexane should therefore be kinked instead of straight (Fig. 34a, b). Molecules which do not form part of a crystal lattice, but can freely move about in the gaseous or dissolved state are subject to the so-called *free rotation* of the groups around the direction of the valence lines. In Fig. 34a a rotation would not give rise to a new structure. In kinked chains, however, the free rotation means that, for instance, group 1 in Fig. 34b needs not necessarily lie in the plane of drawing with 2 and 3. It can be located anywhere on the perimeter of a cone which has its top in group 2 and whose top angle is the valency angle. Among these possibilities there is one special case, in which groups 4 and 5 are turned 180°, thus resulting in a ring-shaped model. It is not difficult to see, that this can easily lead to cyclic compounds. Fig. 34c shows why, preferably, rings of 5 or 6 atoms are formed: the supplement (70°.5) of the valency angle is contained somewhat less than 6 and somewhat more than 5 times in 360° (5·70°.5 = 352°.5; 6·70°.5 = 423°). The different forms which a molecule can assume are called its *constellations*; so Fig. 34b and c represent two different constellations of the same molecule hexane.

Besides carbon, other atoms can also occur in the ring (heterocyclic rings). Let us here briefly discuss the example of sugar, which is so important in biology. The monosaccharides which formerly were considered as "open" chains (Fig. 35a) have been shown to contain a heterocyclic ring with an oxygen bridge. In glucose this is usually a 1–5-bond, often represented in the manner of Fig. 35b. The formula, however,

is not true to reality, since the C-O-distance in it is unduly large. HAWORTH (1925), therefore, writes sugar as an equilateral hexagon or pentagon, according as to whether the oxygen bridge is situated between the carbon atoms 1–5 (derivatives of pyranose) or 1–4 (derivatives of furanose). Figures 35c and d represent the glucose pyranoses. With the aid of the distance rules (see Table III), the dimensions of a glucose molecule can be calculated. For example, on the assumption that the ring is

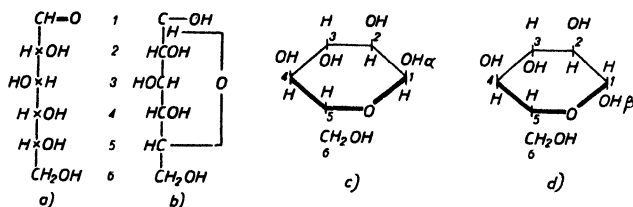



Fig. 35. Glucose. *a*) Aliphatic, *b*) heterocyclic structural formula; *c*, *d*) α- and β-configuration according to HAWORTH.

completely in one plane and represents an equilateral hexagon, the axis drawn through the C-atoms 1 and 4 has a length of $2 \cdot 1.54 + 2 \cdot 1.49 = 6.06$ Å. This value is only approximate, because, to begin with, the hexagon is not completely equilateral on account of the somewhat smaller diameter of the O-atom, and further, the C-atoms, as well as the OH-groups represented by the O-atoms do not lie strictly in the plane in which the distances are measured so that, instead of the distances C-C and C-O, only their projections contribute to the length concerned. If all this is taken into account, the smaller value of 5.15 Å is obtained, which is also derived from X-ray analysis. Fig. 36 shows the far-reaching similarity between the present structural formulae (Fig. 35c) and the molecular models. The former no longer represent arbitrary schemes, but rightly proportioned projections of the molecular structure on a plane.

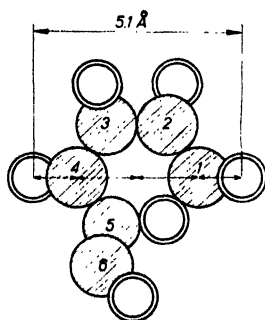



Fig. 36. Molecular structure of glucose (from MEYER and MARK, 1930). C-atoms hatched, O-atoms surrounded.

According to the aliphatic manner of writing (Fig. 35a) glucose contains four asymmetric C-atoms (x), since only the CH_2– and the C = O group have a symmetry plane. As a result of the ring formation, however, the 1 C-atom of the carbonyl group becomes also asymmetric. For that reason two different configurations of the heterocyclic ring are possible; they are called α- and β-glucose (Fig. 35c and d) and are distinguished by their optical rotation (β shows the smaller rotation). It is seen that the β-glucose shows a regularly alternating distribution of the H- and OH-groups on both sides of the ring, while in α-glucose the hydroxyl groups at the 1 and 2 C-atoms are neighbours.

With β-glucose it is possible to lay a second bridge between the 1 and the 6 C-atoms by dehydration (laevoglucosane); in α-glucose this is impossible. This proves that in β-glucose the OH-group of the 1 C-atom lies on the same side of the ring as the one of the 6 C-atom.

The α and β positions of the OH-groups at the 1 C-atom are of fundamental importance for the understanding of the structure of disaccharides and high-polymer

carbohydrates. In disaccharide formation a 1-4-bridge between two glucose rings is formed by loss of one molecule of water. Now it is easy to see that in the case of the α-position the two rings can simply be joined directly, whereas in the case of β-position one of the rings must first rotate through an angle of 180° around its 1-4-axis in order to bring the two OH-groups which are to react into a neighbouring position.

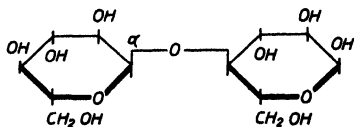
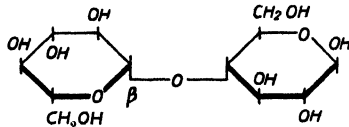



Fig. 37a. Maltose Fig. 37b. Cellobiose
Disaccharides from glucose

Both cases are realized in nature; in the first case maltose is formed and in the second cellobiose, the disaccharide unit of the cellulose chain (Fig. 37). In maltose the two glucose rings can be made to coincide by a simple translation, whereas in cellobiose this requires a digonal axis. The cellobiose molecule possesses therefore a higher degree of symmetry, since the coincidence must be achieved by a combination of a translation and a rotation.

The bond pictured in Fig. 37a is designated as α-glucosidic and the one in Fig. 37b as β-glucosidic. Instead of sugar molecules, all kinds of different molecules containing hydroxyl groups can combine with glucose according to both these schemes, which are then distinguished as α- and β-glucosides respectively. This distinction is not only interesting and important from the point of view of molecular morphology (structural chemistry), but is also of great importance in physiology. In fact, the α- and β-bridges are broken down by quite different ferments. For the hydrolysis of maltose we need an α-glucosidase, which is not capable of splitting cellobiose, while, conversely, β-glucosidases can attack cellobiose but are inactive with respect to maltose. It seems that in plants the reserve substances, which must be quickly mobilized when required, are more often built according to the α-type (saccharose, starch), while glucosides, which cannot be used directly as reserves (e.g., amygdalin) and cellulose, are β-glucosides. This example shows that in the end the problem of enzyms is also of a morphological nature. To be able to distinguish between an α- and a β-bond, they must possess a quite specific structure. Without a knowledge of this structure, it is unlikely that the riddle of organic catalysis will be solved (Mittasch, 1936). The well-known comparison of the lock and the key is not only a symbol, but substrate and ferment must fit together in the strict sense of the word as two parts which are adjusted morphologically to each other.

c. *Structure of Phase Boundaries*

Surface tension. The regions containing phase boundaries are always inhomogeneous. One can only speak of homogeneous phases if in comparison with their surface they are so extended that all surface effects can be neglected.

These inhomogeneities are best known in liquids, where they manifest themselves as surface tension; but they also occur, although less markedly, at the surface of crystal lattices or at the boundary of gaseous phases. The surface tension of a liquid is caused by the fact that the molecules in the bulk of the phase are surrounded on all sides by similar molecules, while in the phase boundary this only occurs on one side. If, by way of example, one considers a boundary layer liquid-gas, the attractive forces of the small number of gas molecules available can on a first approximation be neglected; therefore in the surface the molecules of the liquid are surrounded by a field of cohesive forces quite

different from that in the bulk of the phase. As Fig. 38c shows, the cohesive forces acting on a molecule at the surface do not cancel each other. The particles are therefore attracted by the bulk of the liquid. It will yield to this attraction as far as possible and to some extent decrease its distance from the deeper lying molecules. This results in an increase in density, of which a rough outline is given in Fig. 38d. In this way a surface "skin" is formed, which on its inner side merges into the area of the homogeneous liquid.

The surface skin possesses a certain firmness because its molecules cannot move as freely as in the ideal liquid. This firmness can be determined by stretching a lamella of the liquid suspended in a

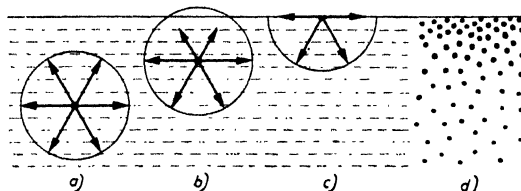



Fig. 38. Inhomogeneity of the phase boundary liquid/gaseous. Cohesive forces *a*) symmetrical, *b*) asymmetrical, *c*) directed towards inward; *d*) Scheme of the inhomogeneous arrangement of molecules (strongly exagerated as the compressibility of liquids is very small).

frame by means of a movable bar, and by measuring the weight needed to break the film. This weight is independent of the thickness of the lamella, but is a linear function of the length l of the bar, since a lamella which is twice as broad can carry twice the weight. The firmness of the surface, therefore, refers to the unit of length, 1 cm, and the force which is capable of rending a lamella surface 1 cm wide is called the surface tension σ of the liquid. As both the surface in front and that at the back of the lamella must be broken, the force $p = 2\,\sigma\,l$ (Fig. 39).

Instead of the more accurate methods of surface tension measurements with the aid of capillary rise or stalagmometry (HÖBER, 1922, p. 154), the much more primitive breaking method has been mentioned here, because the definition of surface tension is founded on it and it demonstrates in a simple way its dimension as force/cm. Surface tension, therefore, is not tension in the ordinary sense, for otherwise its dimension ought to have been force/cm^2. The difference between these two quantities can be seen from the scheme given in Fig. 40. In order to rend a plane the cohesive forces must be overcome along a line only, whereas in the case of a rod the force has to be applied to a plane. Therefore, Fig. 40a and b represent pictures of the definition of surface tension (force/cm) and cohesion tension, or pressure (force/cm^2) respectively.

To understand this better, let us compare the surface tension and the cohesion tension of water. For water at 15° C., σ amounts to 7.30 mg/mm which in absolute units is 71.6 dynes/cm. In order to measure the cohesion pressure or inner pressure (FREUNDLICH and LINDAU, 1932), one must tear apart planes of water in which the molecules cannot change position with respect to each other, for example a film of water between two hydrophilic pistons. Such experiments, however, do not yield

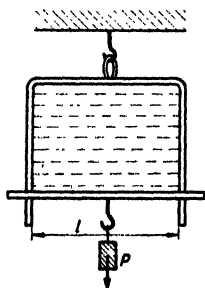



Fig. 39.

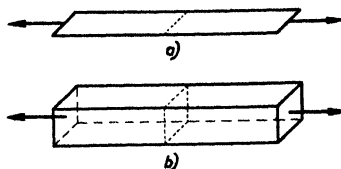



Fig. 40

Fig. 39. Measurement of the surface tension of a lamella (from LECHER).
Fig. 40. *a*) Surface tension (force/cm); *b*) cohesive tension (force/cm^2).

reliable results. The cohesion tension must also be overcome when water is torn from the cell wall in a desiccating cell. According to the osmotic measurements of RENNER (1915) and URSPRUNG (1915) with fern annulus cells this cohesion tension amounts to 300 to 350 atm. From the heat of vaporisation of water, however, the much larger value of about 10^4 atm is derived (LECHER, p. 60). In absolute units this corresponds to an order of magnitude of 10^{10} dynes/cm^2. Since the surface layer of water has a thickness of at least 3 Å (the diameter of the water molecule is 2.78 Å), about $^1/_3 \cdot 10^8$ of these layers are needed to account for this tension. The order of magnitude of this result is correct: multiplying the surface tension of 71.6 dynes/cm of a monolayer by $^1/_3 \cdot 10^8$ we obtain about $^1/_4 \cdot 10^{10}$ dynes/cm^2.

The product of surface tension and area has the dimension of energy: cm^2 force/cm = force·cm = energy. Instead of surface tension the notion of *surface energy* is therefore often used. If much work has to be done to increase the surface, as for instance in water or other liquids with many OH-groups in contact with air, the surface energy is large (see Table VI).

Yet it is impossible to disperse water in ethyl alcohol or other liquids with which it is miscible, in the form of drops, because the water molecules can be transferred to the surrounding dispersing medium without doing any work. The surface tension between two mixing phases is therefore zero; no phase boundary is formed. Nor can a surface energy be attributed to a hydrated solid colloid

TABLE V

SURFACE TENSION OF PROTOPLASM WITH RESPECT TO SOLUTIONS (ACCORDING TO NEWTON HARVEY, 1937)

Naked protoplasts	σ dyne/cm	Medium
Leucocytes (*Lepus caniculus*)	2.0	RINGER solution + serum
,, (*Rana pipiens*)	1.3	,, ,, ,,
Amoeba (*Amoeba dubia*)	1–3	,, ,, , diluted
Slime mold (*Physarum polycephalum*)	0.45	,, ,, , 250 × diluted
Sea-urchin egg (*Arbacia punctulata*)	0.2	seawater
Salamander egg (*Triturus viridescens*)	0.1	pond-water + gum arabic

particle if the water dipoles in the outer shell of the hydration layer have the same mobility as those in the bulk of the water. In that case we are dealing with the situation illustrated in Fig. 20a, i.e., the particle loses its surface and is stably solved in the dispersing medium.

The examples given, show that it is not enough to speak merely of the surface energy of a liquid without specifying the medium in contact with which the surface tension has been measured. The data given in the literature usually refer to the surface tension against air. In cytology, however, we

TABLE VI

SURFACE TENSION AGAINST AIR AT 15° C (HÖBER, 1922, p. 167)

0.25 molar solutions	σ dyne/cm	Relative σ (σ H_2O = 1)
Water	71.6	1.000
Cane sugar	72.1	1.007
Urea	71.6	1.000
Glycerol	71.5	0.999
Acetic acid	66.8	0.932
Ethyl alcohol	66.0	0.922
Ethyl ether (satur.sol.)	53.1	0.742
Ethyl acetate	41.5	0.578
i-Valeric acid	34.9	0.487
i-Amyl alcohol	29.9	0.417

are concerned in the first place with the surface tension of the protoplasm against the nutrient solution or the cell sap (Table V).

The surface tension against air has become of great importance in physiology. As shown by analysis of foams, many substances are accumulated at the surface, which usually lowers the surface tension to a considerable extent (Table VI). On the basis of thermodynamical considerations the theorem of GIBBS THOMSON renders account of this phenomenon by the two following rules: 1. Substances which lower the surface tension of water accumulate at the surface; 2. a small amount of a solute can strongly reduce the surface tension but cannot appreciably increase it.

Hydrophily and lipophily. To-day these relations can easily be understood qualitatively with the aid of simple theories on the mutual miscibility of different types of molecules. Water and ethyl alcohol, for instance, are miscible in any proportion as are also absolute alcohol and ethyl ether. Water and ethyl ether, however, are only miscible to a very small extent. The phase theory contents itself with determining the range of miscibility, without being concerned with the cause of the insolubility. The theory of structure, however, tries to form a notion of the limited solubility of water and ether and vice versa. The reasoning is as follows.

If alcohol and water are mixed, the water molecules will be preferably attached to the kindred hydroxyl groups, more or less according to the scheme of Fig. 41a. In the presence of an excess of water the OH-group is hydrated in much the same way as in Fig. 18 by orienting and attracting the dipoles, be it only to a small extent. Each OH-group, therefore, is surrounded by a water shell designated by the dotted circle in Fig. 41a. The alkyl group on the other hand, tries to escape the water molecules, because it is hydrophobic. It therefore protrudes from the hydration layer if its size allows it, as for instance in butyl or amyl alcohol. In ethyl alcohol, however, the sphere of action of the OH-group corresponds approximately to the length of the alkyl group, so that water dipoles can settle all around the molecule. This explains the unlimited miscibility of ethyl alcohol and water. In the higher members of the aliphatic alcohol series, however, the lipophilic part of the molecular chain predominates, with the result that only a limited number of water dipoles can be attached. If very little water is present, all the hydroxyl groups of the alcohol molecules accumulate round the few water dipoles available (Fig. 41b); in 96% ethyl alcohol, for instance, 9–10 CH_3CH_2OH round each H_2O molecule. This water is bound so strongly, that it can only be separated from the hydroxyl groups by chemical means. As is well-known, absolute ethyl alcohol cannot be obtained by distillation, but only by chemical dehydration.

A still more simple reasoning applies to the miscibility of alcohol and ether (Fig. 41c). Notwithstanding the homopolar character of the ether bridge, i.e., the -O-group, it still has a certain affinity to the OH-group. Consequently, both the hydroxyl group and the alkyl group of the alcohol can enter into some chemical relationship with the two parts of the ether molecule. Not so, when we attempt to dissolve water in ether. It is true that the -O-bridge has a certain affinity to water, but this affinity is small, so that only a limited number of water molecules can be bound by a given number of ether molecules (Fig. 41d). The circumstances are similar to those in 96% ethyl alcohol — but, whereas in that case the number of H_2O molecules round a hydroxyl group could be increased ad libitum, each -O-bridge can only attract a fraction of a water molecule. For that reason, as soon as the amount of water present exceeds a certain limit; the water molecules must cluster together since only very few of them are able to interact with an ether molecule. They accumulate into drops and form their own phase. Conversely, in this phase a few ether molecules can be

dispersed. As will be shown, however, these ether molecules tend to accumulate in the neighbourhood of the phase boundary.

Much the same phenomena are observed in the system phenol/water (Fig. 42).

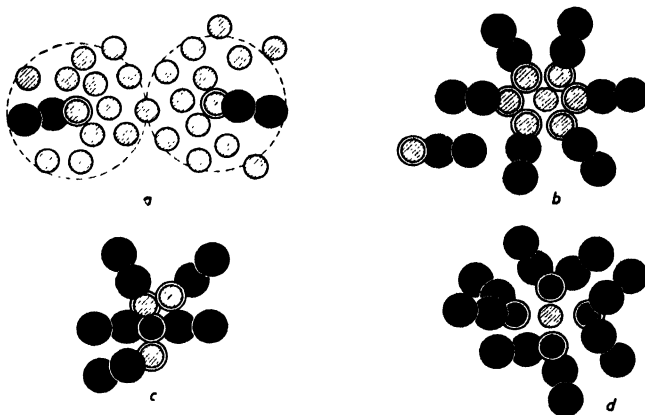



Fig. 41. Solubility. Water molecules and OH-groups hatched, lipophilic groups (-CH_3, -CH_2-, -O- bridges) black. Oxygen groups (-OH and -O-) surrounded. *a*) Ethanol $CH_3 \cdot CH_2 \cdot OH$ and water H_2O (illimited miscibility), *b*) bound water in 96% ethanol, *c*) ethanol and ethyl ether $CH_3 \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_3$ (illimited miscibility), *d*) water in „moist" ethyl ether (very limited miscibility).

If some phenol is added to water it is dissolved. Beyond a certain percentage of phenol, however, two coexistent phases are obtained, which do not mix. Similarly, traces of water are soluble in pure phenol, but if the amount of water is increased one reaches a miscibility limit, beyond which the two phases no longer mix. As shown in Fig. 42, the miscibility does not only depend on the concentration of the two components but also on the temperature. In the region called *miscibility gap* the system is heterogeneous. Here two phases are formed: one consisting of phenol saturated with water and the other of water saturated with phenol. Outside the miscibility gap only a single phase exists, a homogeneous solution with a completely uniform distribution of the phenol and water molecules among each other. On supplying heat, the miscibility of the two components increases, until at a certain temperature (69° C) the miscibility gap disappears. At low temperature the hydration layer of the phenolic OH-group is smaller than the phenylic residue, so that limited miscibility results. With rising temperature the hydration sphere is increased and surrounds at 69° C the whole space of the C_6H_5-group (comparable to Fig. 41a) causing in this way illimited miscibility.

Fig. 42. Diagram of miscibility of the system water/phenol (from ROTHMUND, 1898). Abscissa: from left to right content of phenol in % of weight. Ordinate: Temperature in °C.

To sum up, the decisive factor in the solubility of organic substances in water

is not only the *presence* of hydrophilic (i.e., water attracting) groups, but primarily also the *number* of these groups in comparison with the number of hydrophobic (i.e., water repellent) ones in the molecule. The latter, conversely, are determinant for the solubility in the homopolar solvents for fatty substances, such as hydrocarbons, carbon tetrachloride, carbon disulphide, ether, chloroform, benzene, olive oil, etc., and for this reason are designated as *lipophilic groups*. Table VII gives a survey of the various hydrophilic and lipophilic groups occurring in the organic compounds participating in the construction of the protoplasm. With the aid of this table it is possible in many cases to derive from the chemical structural formula of a substance its solubility in an organic compound.

TABLE VII

HYDROPHILIC AND HYDROPHOBIC GROUPS

	Hydrophilic (lipophobic) dipole character (often tendency to form ions)			*Lipophilic* (hydrophobic) homopolar	
decreasing solubility in water ↓	$—C{\overset{/\!/O}{\underset{\backslash OH}{}}}$	carboxyl	increasing solubility in lipids ↓		
	—OH	hydroxyl		$—CH_3$	methyl
	$—C{\overset{/\!/O}{\underset{\backslash H}{}}}$	aldehyde		$—CH_2—$, $=CH_2$	methylene
	$=C=O$	carbonyl		$—C_2H_5$	ethyl
	$—NH_2$	amino		$—C_3H_7$	propyl
	$=NH$	imino		$—C_nH_{2n+1}$	alkyl
	$—C{\overset{/\!/O}{\underset{\backslash NH_2}{}}}$	amido		$—C_5H_8—$	isoprene group of the terpenes
	$—C{\overset{/\!/NH}{\underset{\backslash OR}{}}}$	imido			
	—SH	sulphydril		$—C_6H_5$	phenyl

Surface films. The lipophilic nature of the alkyl radicals gives an explanation of the lowering of the surface tension reproduced in Table VI. As shown in Fig. 41a, the lipophilic ends of the alcohol molecules protrude to a certain extent from the hydration layer. In their attempt to escape the water dipoles, they tend to approach each other and to accumulate at a phase boundary. It is the hydrophobic nature of the alcohol, therefore, which causes it to accumulate at the surface; it is said to be *surface-active*. This applies, of course, only to those cases where the adjacent phase itself is not hydrophilic. This will practically always hold good at the phase boundary liquid/gas. Fig. 43a shows the arrangement of alcohol molecules in the surface. The lipophilic groups are accumulated at the phase boundary. As their molar cohesion is smaller than that of water with its OH-groups (see Table IV), the surface tension will drop. The molecules of ether or amyl alcohol, in which the lipophilic groups predominate still more, will have a still smaller affinity to water and will lower the surface tension to a greater extent. This explains the first rule in the theory of GIBBS-THOMSON, and also explains why very small amounts are sufficient to lower the surface tension appreciably, since the greater part of the molecules dissolved accumulates at the surface.

For a substance to raise the surface tension it must, so to speak, be more hydrophilic than water. This applies, for example, to sugars, because with their numerous

OH-groups they are able to attract the water strongly. For this reason they do not go into the surface but stay in the bulk of the phase. Their action on the surface tension is due to the fact that the density at the surface is somewhat increased by the attractive forces acting on the water molecules. Clearly, this will only be possible if the concentration of the sugar is very high; in a 0.25 molar solution of cane sugar (the only

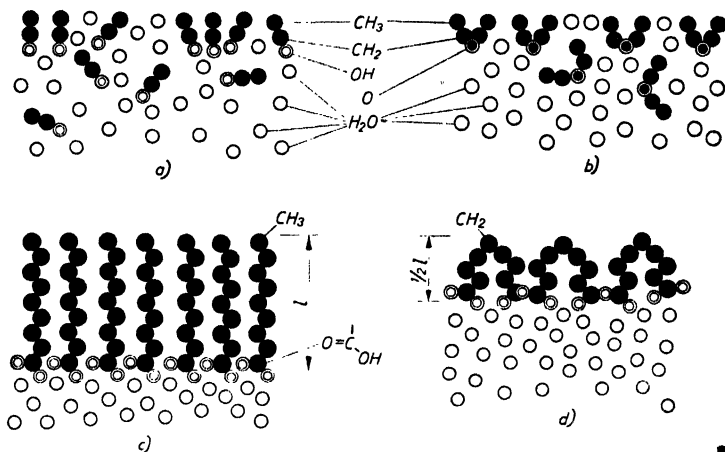



Fig. 43. Molecular surface structure of aqueous solutions. Accumulation in the surface of *a*) ethanol, *b*) ethyl ether. Monomolecular films of *c*) fatty acids, *d*) two-basic acids. o water; hydrophilic groups white; lipophilic groups black; oxygen surrounded.

substance in Table VI which causes a rise in surface tension) the surface properties of the water are changed only inappreciably.

With increasing length of the paraffin chain the hydrophobic character of the alcohol molecules becomes more pronounced, and finally their affinity to water becomes so small that they accumulate quantitatively at the surface. The same applies to the fatty acids. Their aliphatic chains are so hydrophobic that they float on the surface of the water. These floating molecules tend to keep as far apart as possible, in much the same way as the gas molecules in a given volume. They spread over the entire water surface available. The expansive pressure which brings about this spreading can be measured by means of a movable barrier. In the apparatus,originally designed by LANGMUIR in 1917 (LANGMUIR tray), the spreading pressure is transferred from a movable barrier to a torsion balance and measured in dyn/cm with an accuracy of up to 0.01 dynes per cm.

The surface law found with this measuring instrument is similar to the gas law "volume times pressure = constant" in that the product of surface per mole and surface pressure is constant. The floating molecules behave therefore like a gas: the surface density can be increased by reducing the surface. This "surface compression", however, cannot be carried too far; if the surface is reduced below a certain limit, the surface pressure increase becomes steeper than that required by a constant value of the product. At this limit the molecules, which hitherto were freely movable, accumulate into a close-packed *monolayer* (monomolecular film), which has a smaller compressibility. In these films the polar molecules stand up, withdrawing their hydrophobic groups from the water and dipping their hydrophilic groups in the water (Fig. 43c).

The thickness of the film can be calculated from the amount of substance spread on the water and the size of the surface (ADAM, 1930). This thickness corresponds to the length l of the chain molecule (Fig. 43c), and the values found in this way compare well with those derived from the X-ray method in molecule lattices. From the molecular weight of the substance concerned, i.e., from the number of molecules packed in the surface layer, the distance between the chain molecules can be computed; here again the values obtained are similar to those found by X-ray analysis for the distance between the chains in molecule lattices (order of magnitude: 4–5 Å).

Carrying out the same experiment with a two-basic acid, the film thickness found is half that of the corresponding monobasic acid, while the surface occupied is twice as large. For example, the molecular surface of nonyl acid $CH_3(CH_2)_7COOH$ is 25 $Å^2$, while sebacin acid $COOH(CH_2)_8COOH$ fills an area of 57 $Å^2$ (MEYER and MARK 1930). This can be explained by assuming that both the carboxyl groups of the dicarbon acid are dipping into the water, which means that the molecule is bent (Fig. 43d). Such bending is made possible by the free rotation around C-C-bonds.

Although proteins are to a certain extent hydrophilic, they, too, form surface films. Ovalbumin, for instance, spreads on the surface of water in the form of solid skins (DEVAUX, 1935; GORTER and co-workers, 1935). The structure of such films is not yet known in all its details. Molecules of the polypeptide chain type (Fig. 88c) do not stand erect but lie flat on the surface. As a result of their amphoteric nature, their spreading surface is not constant but depends on p_H. It is important to note, that judging from their surface activity not only the skeleton proteins but also the reserve proteins are hydrophobic to a considerable degree (BULL 1947).

The surface structures described in this section are not brought about by primary valencies but merely by cohesion forces. Consequently, the positions of the atoms with respect to each other are not fixed like those in a main valency lattice; a certain mobility exists, of which indications were already found in the ease with which molecule lattices are cleaved and deformed. In surface films, however, the attractive forces are still less pronounced. The molecules in a film containing fatty acids, for instance, are free to rotate about their axis. We might say that surface films are in a state intermediate between the amorphous liquids and the solid bodies with their well-defined regular structure.

d. *Liquid Crystals*

Mesophases. At one time "liquid crystals" played a great part in the discussion of protoplasm structure. LEHMANN (1917) went as far as to attribute life to these remarkable structures. We know now, however, that the unusual properties of "flowing" crystals which on account of their striking birefringence are perhaps better denoted as *anisotropic liquids* are by no means as enigmatic as was formerly believed. For, the structure of liquid crystals is similar to that of the surface films of fatty acids on water. It is usually a matter of chain molecules in parallel alignment, which can be moved with respect to each other in the direction of their axis and are free to rotate about this axis. Whereas, however, the orientation in the surface films is restricted to a small number of monolayers or even to a single monolayer only, the liquid crystals contain oriented structures of microscopic dimensions (deformable crystals, drops, etc.)

The best starting point for a correct understanding of the structure of crystalline liquids is the molecule- or chain lattice represented in Fig. 32. In these lattices the molecules are immovable; the

substance is in the crystalline solid state. If, now, heat is applied to the lattice, the molecules are released at a certain temperature and finally the crystal melts. With increasing chain length, however, the disintegration of the lattice is impeded. Although the mobility of the chain molecules is increased, their parallel alignment is maintained, in much the same way as in a sheaf of pencils in which each pencil can be turned about its axis and shifted with respect to its neighbours, but cannot be turned out of its parallel position. This state is evidently intermediate between the crystalline solid and the amorphous liquid state, because the mobility of the molecules does not refer to all directions in space but is restricted to a single one. We are, therefore, dealing with a state of matter, which is designated as *mesophase* (FRIEDEL, 1922) or *crystalline liquid* (VORLÄNDER, 1936). Since an alignment into loose sheaves is only possible with rod-shaped molecules, only chain molecules can occur as mesophases. If a crystal lattice of isodiametric molecules is dissolved, its molecules become at once independently mobile. With a chain lattice this is not always true, as the pattern is often destroyed in two steps. The first step frees the crystalline bonds between the chain molecules; but there remains some cohesion, which maintains a certain parallelism of the individualized chains, which can rotate and shift along each other as indicated above. In many cases spindle-shaped bundles are formed which are strongly birefringent (compare Fig. 44). It is only by a second step that the crystalline mesophase can be converted into an isotropic amorphous liquid phase, where the molecules become completely mobile.

Fig. 44. Anisotropic liquid aggregates in a sol of benzopurpurin between crossed nicols (from ZOCHER, 1925a).

The transformation of the chain lattice into a mesophase occurs at a well-defined temperature (melting point I) whereas the conversion into an amorphous liquid takes place at a given higher temperature (melting point II).

Compared with solid crystals the optics of mesophases are simplicity itself. As all molecules in the sheaf can be rotated about their axes, no order exists in directions perpendicular to these axes. All directions perpendicular to the axis are, therefore, equivalent in all respects. Consequently, mesophases are usually *optically uni-axial*, and as a rule no isotropic or bi-axial mesophases are observed (ZOCHER, 1925a). In a polarisation microscope between crossed nicols, mesophases will therefore appear completely dark if we observe in the direction of the sheaf axis, whereas they light up in all other directions. According as to whether the refractive index parallel to the axis is larger or smaller than that perpendicular to it, the mesophase is called optically positive or optically negative (compare p. 61).

The ease with which mesophases are changed into an isotropic liquid is a function of the chain length. This is apparent, in particular, from VORLÄNDER's researches (1936). With increasing chain-length it becomes increasingly difficult to attain the amorphous liquid state, because finally the melting point II is such a high temperature that the chain molecules are decomposed before the mesophase is converted into a real liquid. With still greater chain-lengths the substance does not fuse at all, because the molecules are subject to degradation before becoming movable. In this case, therefore, the cohesive forces between the very long chains are stronger than the main valency bonds in the chain molecule: the molecular structure breaks down before the lattice disintegrates. Substances which cannot be changed into the amorphous state, because the *inter*-molecular forces in the lattice or in sheaves (mesophase) are larger than the *intra*-molecular binding forces are called *super-crystalline* (VORLÄNDER). A survey is given in Table VIII; the substances (2) and (3) occur as mesophase at certain intervals of temperature.

Fig. 45 shows a series of molecules of increasing chain-length which corresponds

TABLE VIII

CRYSTALLINE LIQUID STATE (ACCORDING TO VORLÄNDER 1936)

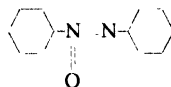
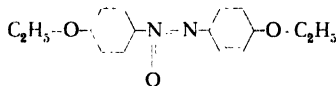
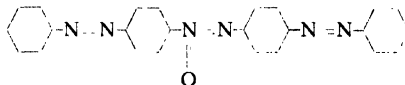
Compare Fig. 45

Increasing chain-length ↓		Solid phase	melt.pt. I ↓ Mesophase	melt.pt. II ↓	Liquid phase
	(1)	Crystalline solid	⇌		amorphous liquid
	(2)	,, ,,	⇌ crystalline liquid	⇌	,, ,,
	(3)	,, ,,	⇌ ,, ,, (supercr.) →		decomposed
	(4)	,, ,, (supercr.) →	decomposed, infusable		
		rising temperature →			

with the general plan of Table VIII. It is striking in this series that the addition of only a single pair of members to the chain results in such radical changes in the physical properties. It is to be noted, that this holds good only for para-substituents in the benzene ring, leading to one-dimensional chain molecules.

1) p-Azoxybenzene
cryst.-solid ⇄ (36°) am. liq.

2) p-Azoxyphenetole
cryst.-solid ⇄ (134°) cryst. liq. ⇄ (165°) am. liq.

C_2H_5–O–C₆H₄–N=N(→O)–C₆H₄–O·C_2H_5

3) p-Azoxy-azobenzene
cryst.-solid ⇄ (226°) cryst. liq.→decomp.

4) p-Azoxy-disazobenzene
cryst.-solid→decomp.

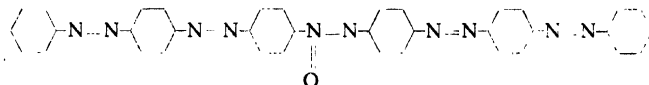



Fig. 45. Series of chain molecules which aggregate to mesophases (compare Table VIII).

Myelin forms. Cytologists are more familiar with the birefringent semi-liquid worms, designated as myelin forms because they were observed for the first time with nerves in myelin sheath (Fig. 153a). When adding water to nerve fibres containing marrow, adventurous threads issue from the nerve sheath. They bend and curl and finally grow into irregular entanglements. The active substance causing these structures is the lecithin in the myelin sheath, for exactly the same phenomena are observed when water is added to isolated lecithin, especially if this is liable to decomposition. Although the myelin forms are particularly striking in organic phosphoric acid compounds, similar worms emerge from the alkali salts of oleic acid when these are wetted. Very beautiful myelin forms were obtained by GICKLHORN (1932a) in the cell sap of the well-known *Allium* epidermic cells by adding of ammonia or sodium hydroxyde (Fig. 46). The variety of shapes in these peculiar structures is well demonstrated by NAGEOTTE's microphotograph atlas (1936, no. 434).

The myelin forms are usually designated as liquid crystals. We should like to point out, however, that there is a fundamental difference between these structures

and the crystalline liquid state mentioned above. For, in the latter we have to deal with a special aggregate state of a uniform substance, i.e., a system consisting of one component, whereas in the formation of myelin forms at least two components take part. In the examples mentioned one of these components is water. It is further essential that the molecules, which here again must have a chain-like structure, are not homopolar as in Fig. 45, but heteropolar. That is, they must contain a hydrophilic and a

Fig. 46. Myelin forms in the epidermal cells of *Allium* (from GICKLHORN, 1932a).

lipophilic pole. The hydrophilic group in oleic acid is the carboxyl group, that in lecithin is the cholin. If the conditions mentioned are realized, myelin forms can occur, provided the molecules are sufficiently mobile.

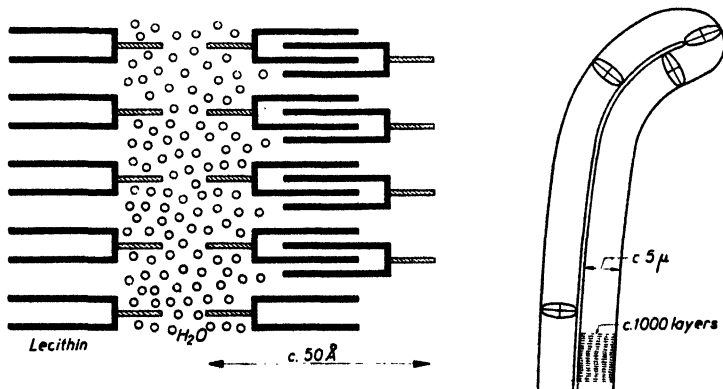
The apparent growth is due to water absorption; it is, therefore, a matter of *swelling*: the hydrophilic groups are surrounded by water, while the hydrophobic groups are drawn away from the surface. The resulting orientation in the case of lecithin is represented in Fig. 47; the lecithin underlying this scheme is a β-lecithin (see Fig. 118) in which the phosphoric acid is attached to the OH-group in the middle of the glycerol molecule. Obviously, the water penetrating into the lecithin causes the molecules to arrange themselves in layers which are similar to surface films, except that it is not a matter of mono- or oligomolecular layers but of huge, microscopically visible structures consisting of bimolecular lamellae. Attributing a length of about 50 Å to the pair of overlapping lecithin molecules (TRILLAT, 1925/27),

Myelin forms of lecithin.

Fig. 47. Submicroscopic structure. Hatched hydrophilic, black lipophilic part of the fork shaped lecithin molecule. – Fig. 48. Microscopic image and optics.

a wall of a myelin tube 5 μ in thickness consists of some 1000 double layers (Fig. 48). As long as not all hydrophilic groups are saturated with water, more water is absorbed, causing the worms to grow further. In course of time the myelin forms therefore penetrate right across the field of view under the cover glass of the microscopic preparation.

It can be proved by optical means that the lecithin molecules in the myelin tubes are perpendicular to the surface. For, in a flowing solution (see p. 63) the lecithin molecules appear to be optically positive. The myelin worms, however, are optically negative with respect to their long axis. It follows that the lecithin chains are perpendicular to the worm axis. BEAR and SCHMITT (1936) mention a formula (p. 61) from which the double refraction $n_e - n_o$ of the cylindrical myelin tube with its optical axis in radial direction can be computed. For the myelin forms of lecithin in RINGER solution the authors find $n_e - n_o = 0.0039$ (SCHMITT and BEAR, 1937). On further absorption of water the lamellar structure of the myelin forms becomes increasingly pronounced. Finally the positive intrinsic double refraction of the molecules is overcompensated by the negative double refraction due to the lamellar texture (see p. 58), and the sign of the myelin birefringence is reversed (NAGEOTTE, 1936).

Fig. 49. Water intercalation between bimolecular lipidic films. Size of the adsorbed water layer with increasing water content. The black points correspond to 0%, 25%, 50%, 67% and 75% of water-content (from SCHMITT and PALMER, 1940).

The absorption of water can be followed by means of X-rays. The dry myelin substances obtained by evaporating the benzene solution give X-ray interferences which correspond to twice the chain-length (lecithin and cephalin 44 Å, sterol 34 Å, sphingomyelin and cerebroside 63–67 Å; SCHMITT and PALMER, 1940). If water is added to these lipids, the X-ray periods are enlarged and so allow of an evaluation of the thickness of the water lamellae formed. It can be seen from Fig. 49 that the original period of 63.5 Å of mixed nerve lipides has become 150 Å at a water content of 75%. This leads to a water layer of 86 Å between the bimolecular lipid layers.

The myelin forms offer a good example of the manner in which complicated microscopic structures can result from a simple arrangement of submicroscopic entities. They show, however, that no coordinated growth is possible as a result of such a process, for the myelin forms "grow" at random aimlessly and choicelessly in the substrate and the final outcome is a chaos rather than an illustration of organized life.

§ 3. STRUCTURE OF GELS

a. *Chemistry of High Polymers*

Polymerisation and condensation. Since about 1920 STAUDINGER drew attention to the fact that in the high polymer natural substances the structural units which can be obtained from them by hydrolysis are interlinked by primary valency bonds (KÉKULÉ bonds). He first proved the correctness of this point of view in synthetic products. Fig. 50 shows some of his polymerisations. It is seen that the monomer molecules always contain double bonds, one of which interacts with another molecule

Monomers | Polymer chains

$H_2C{=}O$ (H—C(=O)—H) | —CH_2—O—CH_2—O—CH_2—O—CH_2—O—CH_2—O—

Formaldehyde (oxymethylene) | Polyoxymethylene

$CH{=}CH_2$ (with phenyl ring) | —CH—CH_2—CH—CH_2—CH—CH_2—CH—CH_2—CH— (each CH bearing a phenyl ring)

Styrene | Polystyrene

$CH_2{=}C(CH_3){-}CH{=}CH_2$ | —CH_2—C(=CH—CH_2—CH_2—C(=CH—CH_2—CH_2—C(= CH—, each C bearing CH_3

$CH_2 = C—CH = CH_2$, with CH_3 on C | —CH_2—C = CH—CH_2—CH_2—C = CH—CH_2—CH_2—C = CH—, each C bearing CH_3

Isoprene | Caoutchouc (polyprene)

Fig. 50. Polymerisation.

and thus links two monomer molecules together. If this process is repeated, long chain molecules are formed whose growth would be theoretically unlimited if the possibility of further addition did not diminish with increasing chain-length and the sensitivity to oxygen (and the like) of the giant molecules formed did not become considerably enhanced. For the present, however, the factors limiting the chain-length will not be considered, and the polymer chains will simply be denoted by "open" formulae. Polymerisation processes are particularly successful if the monomer contains a system of *conjugated double bonds*, as e.g., in isoprene, i.e., if double bonds alternate with single bonds. The terminal double bonds may then give rise to interlinking with those of neighbouring monomer molecules, while the central single bond is converted into a double bond. In this manner *unsaturated* high polymer compounds are formed, such as rubber in the case considered here.

Apart from this type of chain formation, high molecular substances may also be formed by etherification of alcoholic groups (Fig. 51) or by a process of esterification between carboxylic and hydroxylic groups with elimination of water. This way of interlinking is distinguished as *condensation* from the polymerisation of unsaturated compounds. It leads to equally long molecules; the chains are then, however, no more all-carbon chains like those in polystyrene or rubber, but always contain oxygen atoms as interconnecting links. When polyvalent alcohols react with each other, no chain-like but net-like or even spatial giant molecules are formed, such as probably occur in the insoluble huminic acids and in the insoluble portion of lignin. By way of introduction, however, we shall confine the discussion to the somewhat simpler conditions in the high polymer carbohydrates with linear chain molecules.

The high polymer molecules may become so large as to assume the properties of colloid particles. STAUDINGER (1936a) designates these giant molecules as *macromolecules* and the branch of science dealing with their constitution and chemical behaviour as *macromolecular chemistry*.

Polysaccharides. The same principles which apply to the formation of disaccharides (see Fig. 35/37) also apply to the structure of polysaccharides, which are of out-

standing importance for plant physiology. Here too, the monoses are interlinked by 1–4 oxygen bridges with elimination of water, and this polycondensation may embrace a large number of monomer molecules. In *cellulose* the successive links of β-glucose are rotated 180° with respect to each other. In starch, however, the α-glucose residues can react with each other without being rotated (Fig. 51). The cellulose chains have a digonal screw axis as an element of symmetry, contrary to the starch

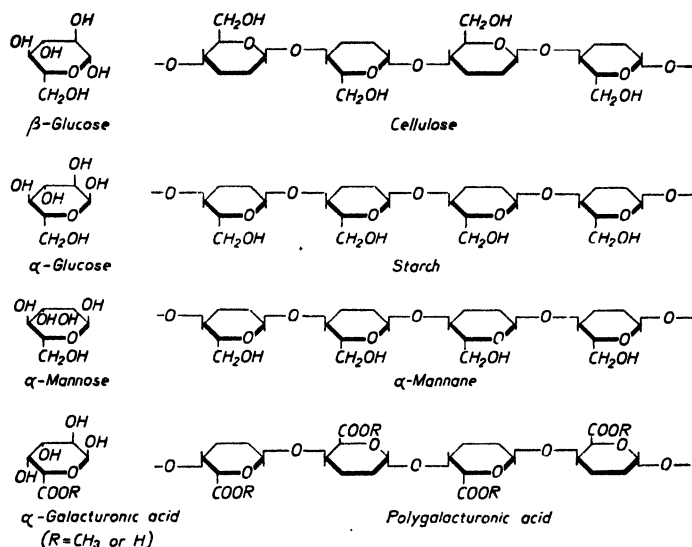



Fig. 51. Polysaccharides.

chains which have not. Consequently the cellulose molecules are more stable and straightened out, whereas the starch molecules tend to become more convolute because it is less symmetrical. This morphological difference is doubtless one of the reasons for the difference in behaviour between starch and cellulose. Possibly it is also responsible for the tendency of the starch molecule towards branching (see Fig. 156b). The mannanes occurring in corozo nut and in the rhizomes of *Amorphophallus konjak* (see Fig. 159) can be derived in a similar way from mannose as starch and cellulose from glucose. The two monoses differ only in the different position of the H- and OH-groups at the second C-atom. For the chain in mannane from corozo nut, localised in the cell wall, MEYER and MARK (1930, page 168) assume β-glucosidic bonds, while it seems likely that to Konjak mannane, being an intracellularly deposited reserve substance, a starch-like structure with α-glucosidic bonds should be assigned.

It is very important that the *pectic substances* which are held responsible for the coherence of plant tissues also contain polygalacturonic acid as a basic material having the structural principle of polysaccharides. Here the $-CH_2OH$ side-chain of the monose ring is replaced by the carboxyl group $-COOH$. The pectins are therefore capable of salt formation. Polygalacturonic acid is soluble in water, its Ca-salt, however, is not so that this polyacid can be precipitated by Ca-ions. Part of the carboxyl groups is esterified with methanol (DEUEL, 1943). Interestingly, the methylation does not interfere with the solubility in water, because methyl groups bound to oxonium oxygen lose their lipophilic character and become hydrophilic.

The monomer of the pectic acid is the α-galacturonic acid. As in α-galactose the hydroxyl groups of the first and the fourth C-atom are not situated on the same side of the pyranose ring (Fig. 51), the α-glucosidic linkage causes a rotation of succeeding chain members. In crystalline sodium pectate the screw axis is not twofold as in cellulose, but a threefold screw axis (PALMER and HARTZOG, 1945). The crystallising tendency of pectic substances is much smaller than that of cellulose; in the plant it occurs in the amorphous state only (WUHRMANN add PILNIK, 1945).

The *pentosanes* which partly fall under the heading of hemicelluloses have a similar structure as the polysaccharides already described except for the absence of the side chains, i.e., the sixth C-atom. If in cellulose resp. polygalacturonic acid this group is replaced by H, we obtain the *xylane* chain resp. a *polyarabane*.

The polysaccharides demonstrate strikingly how slight morphological variations of one and the same structural principle may give rise to substances which behave quite differently from a physiological point of view.

Chain length of high polymers. According to STAUDINGER, all high polymer chains terminate in end groups. Unfortunately, so far the terminal groups of none of the high molecular natural substances are known; the chains are therefore preferably written in "open" formulae (Fig. 51). Contrarily, in relatively short synthetic chains the end groups and thus the molecular weights of the products can be determined. If foreign atoms, such as, for instance, iodine form the terminal groups, such determinations can be easily performed. If, however, the chains are terminated by OH-groups, the accuracy of this so-called end group method diminishes rapidly with increasing chain length. In polyoxymethylene dimethyl ether this method can be successfully applied up to a degree of polymerisation of about 100. The methods of freezing point depression and rise of boiling point, commonly used in molecular weight determinations in low molecular substances cannot be applied to high polymers since the effects are too small.

On the other hand, the molecular weight and thus the chain length of high polymers can be measured by osmotic means, in which case it must be taken into account that VAN 'T HOFF's law does not apply rigorously to molecules of so great a volume. Corrections similar to VAN DER WAALS' b-correction in the equation of state of gases must therefore be introduced (SCHULZ, 1936). A method introduced by STAUDINGER is based on the fact that the specific viscosity of chain molecules (i.e., the viscosity increase which is imposed upon the solvent by the solute), within a certain range of molecular weights is approximately a linear function of the chain length. In addition to osmometry and viscometry we mention in particular SVEDBERG's ultracentrifuge for the determination of the degree of polymerisation of high polymer natural substances, whereas X-ray analysis is not suitable for this purpose (see p. 67).

According to STAUDINGER the experimental data available lead to the following conclusions regarding the molecule type of cellulose (Fig. 51). If some 10 glucose residues are linked together to form a chain, one obtains easily soluble cellulose products which owing to their particle length of 50 Å already exhibit slightly colloidal properties. Compounds of this kind are known as degradation products of cellulose termed cellodextrines or γ-celluloses. If the number of chain links increases to 100, β-celluloses are obtained which are soluble in 10% sodium hydroxyde without swelling, to form viscous sols. Not before the degree of polymerisation surpasses 100 and approaches 800 do we obtain the so-called α-celluloses which are no longer attacked by 1 % sodium hydroxyde and which find application in the cellulose industry (rayon, cellophane). They slowly dissolve under swelling in 10% NaOH and yield viscous "gel-solutions". Native cellulose has a still higher degree of polymerisation; if dissolved in SCHWEIZER's solution with complete exclusion of oxygen one can calculate from the viscosity a degree of polymerisation of about 2000 for the fibre cellulose of linen, hemp, ramie and others. The values determined from the viscosity can be checked osmometrically up to a degree of polymerisation of about 1000

TABLE IX

POLYMER HOMOLOGOUS SERIES OF CELLULOSE

	Degree of polymerisation	Chain length	Mechanical properties
Oligosaccharides γ-cellulose	1–10	– 50 Å	Pulverisable crystal powder
Hemicolloid cellulose β-cellulose	10–100	50–500 Å	Short-fibrous pulverisable powder
Mesocolloid cellulose α-cellulose (rayon)	100–500	500–2000 Å	Fibrous, strong
Native cellulose, α-cellulose (fibre cellulose)	500–2000	0.25–1 μ and more	Long-fibrous, very strong

(STAUDINGER, 1936 a, b); beyond this limit extrapolation is carried out according to the linear viscosity rule. Whether this applies to the whole range from 1000 to 2000 chain links cannot be decided. Furthermore, some doubt has been put forward whether it is at all possible completely to disperse chain molecules of such highly polymeric substances in a micromolecular solvent (LIESER, 1940,1941). On the other hand, it is not certain that native fibres do not contain still longer chains, which may be degraded on dissolution in cuprammonium. The value of 2000 for the degree of polymerisation of the fibre celluloses is, therefore, not reliable; it represents, however, the only value which can be determined at present experimentally and to which, for the time being, we must refer. Its magnitude is already quite impressive, since a degree of polymerisation of 2000 corresponds to a chain length of 1 μ, each glucose residue measuring 5 Å. The cellulose molecules possess therefore microscopical lengths. Nevertheless, they remain invisible because their thickness is amicroscopic.

Chain molecules of a given structural type but different chain lengths are called a *polymer homologous* series. The polyglucosanes mentioned represent the polymer homologous family of the celluloses. In such a series the physical properties change with increasing molecular weight according to certain laws. Table IX gives data for cellulose. Not only does the solubility decrease and the viscosity of the solutions increase, but the fibrous character and the capacity for film formation which are of particular importance for biology become increasingly pronounced beyond a certain degree of polymerisation.

It is only from the low molecular members of a polymer homologous series that uniform substances of definite molecular weight can be obtained by recrystallisation, fractional precipitation, etc. In the members of higher molecular weight this is no longer possible. In the series of paraffins in particular it has been found, that fractionation gives mixtures of substances of molecular weights which are only approximately equal. The determination of the degree of polymerisation yields therefore only an average value; the actual chain lengths are spread more or less around this value according to the method of fractionation. Such mixtures are called *polymer uniform* substances by STAUDINGER. Whether the high polymers occurring in nature are also polymer uniform, or whether life always builds chains of exactly the same length cannot be decided at present. The latter, however, seems unlikely.

(ACCORDING TO STAUDINGER, 1936b, 1937a)

Capacity of film formation	Solubility in 10% NaOH	Viscosity in 1% SCHWEIZER solution	Deviation from HAGEN-POISEUILLE law in 1% solution
None	Easily soluble without swelling	Solution of low viscosity	None
Small	Soluble without swelling	Viscous solution	None
Large	Slowly dissolved under swelling	Viscous "gel-solution"	Small
Very large	Strong swelling almost insoluble	Highly viscous "gel-solution"	Strong structural viscosity!

Although the representatives of a homologous series behave quite differently from a physical point of view, they show the same or at least a very similar chemical behaviour, in conformity with their uniform structure. For instance, the alcoholic OH-groups of all representatives in Table IX and, further, those of the polysaccharide molecules shown in Fig. 51 and even those of the polygalacturonic acid chains (SCHNEIDER *et al*, 1936) can be etherified and esterified (methylated, acetylated, nitrated, etc.) without measurable change in the degree of polymerisation. The polymer mixture formed in this way from the polymer uniform substance concerned has the same average chain length as the original material (it is "polymer analoguous", STAUDINGER, 1936b). On esterification, the cellulose chains lose their polar, hydrophilic properties, acquire a more homopolar lipophilic character and on account of their solubility in organic liquids are then more accessible to osmotic experiments.

b. *Structural Viscosity*

Anomalous flow. The four fractions of the polymer homologous cellulose series yield colloid solutions of an entirely different nature. STAUDINGER divides them into "almost, meso-, hemi- and eu-colloid" (Table IX). In the two former cases the chain molecules in 1% SCHWEIZER solution are completely solvated, i.e., completely surrounded by molecules of the solvent, and free to move as in real solutions. Their colloid character results merely from the fact that the solute molecules possess in one dimension a more than molecular length, attract a large amount of solvent and thus increase the viscosity. STAUDINGER denotes this state as "sol-solution". From a degree of polymerisation of about 100 onwards, however, a 1% SCHWEIZER solution can no longer completely solvate all the chain molecules, and the solute molecules hamper each other's Brownian movement. They are not completely dissolved but are in a state intermediate between the solid and the liquid one. At the highest degree of polymerisation detectable, this interaction of the giant chains with 2000 links becomes so large, that the fibre cellulose is only dissolved very slowly. Solutions in which the chain molecules are hampered in their Brownian movement for want of solvent were called "gel-solutions" by STAUDINGER (STAUDINGER and SORKIN, 1937b). Based on the phenomena of capillary flow there exists a reliable method to decide at what concentration or at what particle size the particles in a colloid solution begin to disturb each other; to wit HAGEN-POISEUILLE's law

$$q = \frac{\pi r^4 p}{8 \eta l} t,$$

where q is the amount of liquid flowing through a capillary of radius r in a time t under the influence of a pressure gradient p/l. In this formula the viscosity η is independent of the pressure gradient p/l.

This no longer applies when the colloid particles in the solution have an influence on each other's motion. In this case the viscosity depends on the pressure gradient: $\eta = f(p/l)$, in such a way that the viscosity decreases with increasing pressure gradient. This can be explained by observing that in these solutions the *elastic properties* of the solid substance are not completely eliminated, since the particles, instead of being fully dissolved, enter into some sort of relation with each other. With increasing pressure gradient in the capillary these elastic forces are progressively counteracted. For this reason, in colloid solutions with long chain molecules the chains which are originally present in a random and disordered arrangement will be orientated parallel to the direction of flow, and thus the forces resisting the flow which are responsible for the viscosity will be decreased. According to Table IX such deviations from HAGEN-POISEUILLE's law are observed in the case of cellulose of polymerisation degrees exceeding 100. Since the anomaly of flow is caused by the mutual positions of the colloid particles, it has been designated as *structural viscosity* (OSTWALD, 1925; PHILIPPOFF, 1935).

Structural viscosity of the protoplasm. It is of great importance to prove the existence of anomalous phenomena in the flow of protoplasm, since this serves to decide whether living matter represents a dispersoid liquid with particles independent of each other as is often supposed, or whether there exist mutual spatial relations between the structural units. In order to solve this question, viscosity measurements with different pressure gradients must be carried out with the same object.

Fig. 52. Structural viscosity of the cytoplasm of *Chara fragilis* (according to PFEIFFER, 1936). Abscissa: pressure p in cm H_2O. I Cytoplasm at 21°C, II at 12°C; III glycerol at 21°C. Ordinate: Viscosity η in % of the original value.

Since plasm cannot be made to flow through a capillary like a liquid, PFEIFFER (1936) sucks naked protoplasts (so-called gymnoplasts from the decomposing fruit pulp of *Physalis*, *Solanum* or *Juniperus*, of *Allium* epidermic cells, etc.) through a capillary under a given pressure difference which can be read from a manometer. At the same time he measures the viscosity by following the Brownian movement of particles (dyed by means of chrysoidin) which are embedded in the plasm drops (PEKAREK, 1932). In Fig. 52 the viscosity η is plotted against the pressure gradient p for plasm drops from the cells of *Chara fragilis*. The viscosity decreases rapidly with increasing pressure (measured in cm H_2O), whereas in normal flow of glycerin η remains practically independent of the pressure. This experiment shows clearly that protoplasm is not a sol-like liquid but represents an *elastic* "gel-solution".

c. *Gel Structure*

Gel frame. If the coherence between the individual colloid particles becomes still more pronounced than in the "gel-solution", gels are formed in the end with a more or less fixed shape and distinctly *elastic* properties. Of course there exist all kinds of transitions between the gel-solutions in which the elastic coherence of the particles can only be proved by testing them on structural viscosity and the real gels whose units are more or less fixed in their mutual positions. Those gels that become liquid on shaking and solidify again at rest form a typical intermediate stage. This remarkable phenomenon is due to the same effect as the decreasing viscosity at increasing pressure; it is designated as *thixotropy* (compare FREUNDLICH, 1942). Colloid silicic acid and gelatin, for instance, can occur as thixotropic gels at suitable concentrations.

If spherical colloid particles cluster together to a gel, the result is a rather compact gel and from Fig. 53a it is obvious that such a structure can only be formed at a relatively high concentration of the solute. With sols containing long chain molecules, however, a fixed mutual position, i.e., a *structure* is achieved much more easily. At concentrations as low as a few per cent of a long chain high polymer, the chain molecules can combine into a loose structure, as represented by Fig. 53b. Such a colloid already possesses a structure, although a very loose one, which as yet can easily undergo a plastic deformation. It also possesses a certain elasticity since the points where the chains are interlaced represent some sort of fixed points. As will be shown, these can be due to various kinds of forces. Since in biologic colloids it is often difficult to decide upon their nature, I have proposed to characterize them by the neutral name *points of attachment* or *junctions* ("Haftpunkte") which does not imply anything about the kind of bonds (FREY-WYSSLING, 1935 b; 1936 a; BONNER, 1935, p. 404).

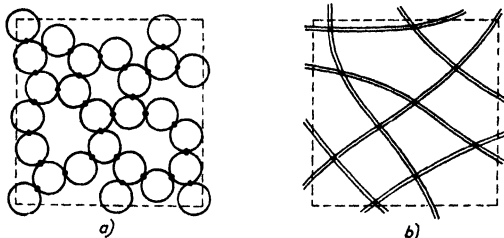



Fig. 53. Submicroscopic structure formation *a*) with spherical, *b*) with filiform particles.

In Fig. 53 the junctions are marked by black dots. Obviously in a gel with chain molecules many fewer junctions are needed to build up a structure than in the case of spherical colloid particles. A gel built up by high polymer chains can therefore contain *up to* 97 % *of water* (SEIFRIZ, 1938) *and yet possess a structure*. This fact is very important for the understanding of protoplasm structure, since the water content of living matter is always astonishingly high.

In Fig. 53b further chains can be plaited in at will; the number of junctions will then increase, and the result is a more solid gel structure. The plastic properties of the structure become less pronounced at the same rate, while the elastic properties become more pronounced. Thus the model of a gel structure projected here comprises all the states ranging from gels very rich in water to those very poor in water, as they are characteristic for the protoplasm.

Limited swelling. In the swelling process the swelling medium penetrates into the interstices available in the gel structure and widens the framework. It is clear that the permeating liquid should show chemical affinity to the chain molecules concerned. Thus the lipophilic molecules of rubber and polystyrene swell in the lipophilic benzene, while the hydrophilic cellulose swells in water. Whether in this process the framework of the gel goes to pieces, i.e., whether the gel is dissolved depends on whether the junctions present can be disrupted. If the bonds are of the type of cohesive forces and the solvent present is capable of completely solvating the chain molecules, the gel structure can be abolished and change into

Fig. 54. Limited swelling of polystyrene (according to STAUDINGER, 1936a).

a gel-solution in which the particles possess greater mobility. This happens, for instance, in the swelling of fibre cellulose in cuprammonium. Limited swelling, therefore, always indicates that the chain molecules can only be solvated to a certain extent.

Sometimes main valency bonds may be among the junctions. For example, as shown by STAUDINGER (1936a), polystyrene with a degree of polymerisation 1700 is soluble in benzene, but on the addition of traces of divinylbenzene (0.002%) it is converted into a product showing limited swelling in benzene (Fig. 54). In the same way chains of methylcellulose can be interlinked by dicarbon acids (TAVEL, 1939) or chains of polygalacturonic acid (pectic acid) by epioxides (DEUEL, 1947). When main valency bonds occur between the chain molecules, even the most suitable solvating medium imaginable is no longer capable of destroying the gel structure. Notwithstanding remarkable swelling (e.g., a 30 fold increase in volume) the framework of the chains is preserved. It is possible that cellulose also contains a few of such main valency bridges (see, for instance, ESCHER, 1936; LIESER, 1940; MEYER, 1940 a). These would limit the swelling and would have to be degraded chemically when cellulose is dissolved in cuprammonium.

Generally can be said that limited swelling occurs when certain junctions of the gel frame (cohesive or main valency bonds) cannot be loosened.

"Phase build" of gels. In the case of a sol one can (if necessary) speak of a "dispersed phase" distributed in a dispersing medium, although difficulties arise which have already been mentioned on p. 13. With sols containing chain molecules instead of colloid particles in the sense of the classical theory of dispersoids, to uphold the concept "phase" is decidedly wrong. For, according to the definitions in phase theory, separate molecules may not be characterized as phases. With gels the conditions are much the same. In a chain framework not only is the designation "dispersed phase" not permissible, because regions with a thickness of molecular dimensions are not homogeneous phases, but also the concept "dispersing medium" becomes questionable. Consider a gel consisting of equal percentages of chains and water; a projection of the structure then gives the impression that the water is distributed as a "dispersoid" in closed compartments (Fig. 55a) whereas, conversely, in a cross-section of the gel the cross-sections of the chain molecules appear as "dispersed" particles distributed in the liquid (Fig. 55b). In reality, however, neither of the two partners is "dispersed" relatively to the other, for they both fill the available space continuously.

A gel of chain molecules is therefore not a two-phase system but a *single* undivided phase. It is not only microscopically homogeneous and optically empty, but also homogeneous from a physico-chemical point of view. As in the case of real solutions, if small volumes are considered, one always finds the same composition, with the sole difference that the volumes contain only parts of chains instead of whole molecules (Fig. 55b). Thus gels with a skeleton consisting of separate chain molecules are *one-phase* systems. As in the case of mesophases this state deserves a nomenclature of its own. It will be designated as *pseudophase*, especially in view of the fact that often not all junction bonds are identical, so that the condition of homogeneity is not strictly satisfied. This applies in particular to protoplasm (see p. 97).

In concentrated gels the chain molecules show a pronounced tendency to cluster together in strands or rods. In such cases the parallel arrangement can become so pronounced that here and there the chain molecules combine to form a chain lattice.

The length of the crystal lattice in the direction of the chain axis need not be the same as the length of these chain molecules; the chains can protrude from the end planes of the crystalline rods (GERNGROSS, HERRMANN and co-workers, 1930, 1932), continue further and eventually enter again into another region of lattice order, as has

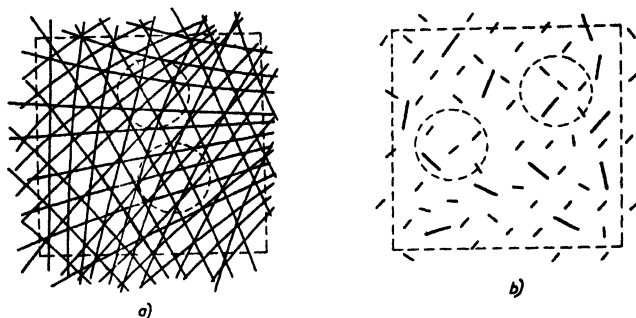



Fig. 55. Gel framework. *a*) Projection, *b*) section across the framework. Districts to be compared surrounded.

been indicated schematically in Fig. 56a. The more complete the average parallel arrangement of the chains, the greater the probability of the occurrence of crystals (Fig. 56b). In this case the gel is no longer a one-phase system: the regions of lattice order form a homogeneous phase in contrast with the pseudophase formed by the mixture of the unordered chains and the surrounding liquid.

Hence, from a structural point of view there are two kinds of gels: 1. one-phase gels whose framework consists of very long chain molecules interlinked at the junctions (pseudophases) and 2. two-phase gels with a crystalline and a noncrystalline (amorphous) phase. Instead of the fine chain framework of the one-phase gels we have then to deal with a much coarser rod framework.

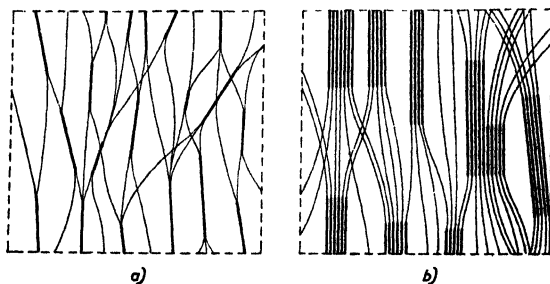
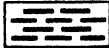



Fig. 56. Ordered districts in a gel framework. *a*) Locally parallelised chain molecules, *b*) local formation of a crystal lattice.

Dispersity series. After having derived the structure of gels from the special form of the high molecular chain molecules — thus starting from below, that is from the amicroscopic domain — we will now try conversely to advance into the submicroscopic domain of gels from the macroscopic and microscopic regions. In colloid chemistry the concept of colloid particles is usually derived from macroscopic

particles with the aid of a *dispersity series*. The particle size in this series decreases steadily to microscopic dimensions and finally the step to invisible submicroscopic dimensions is made. A further and last step in the direction of proceeding dispersion leads from the colloid range into the amicroscopic dispersions of true solutions (Table X).

TABLE X

DISPERSITY SERIES

Order of magnitude of structural unit	Corpuscular disperse systems	Reticular disperse systems
Macroscopic	Gravel Sand	Liane undergrowth; veil of aerial roots
Microscopic	Dust	Wad of threadlike algae
Submicroscopic	Clay	V_2O_5 gel, cell wall
Amicroscopic	Salt solution	Methyl cellulose; cytoplasm(?)

When making a similar dispersity series for gels one must start from *reticular* instead of corpuscular systems. The frequency of such systems in biology is surprising; one comes to the conclusion that network systems of all dimensions are typical for living matter!

The entanglement of lianes in a virgin forest is a macroscopic network system (Fig. 57). As an example of fibre network we mention the veil of aerial roots of *Cissus* lianes in a tropical forest: thin filiform roots with a length of several metres hang slackly from the branches. They form as it were a fabric in the air, although none of these aerial roots has grown together. In moving air this entanglement of roots behaves as a coherent mass because neighbouring filaments impede each other's free movement. Plenty of other, still finer macroscopic network systems may be mentioned: skeletons of vascular bundles of leaves, succulent sprouts and fruits (Fig. 58), skeletons of sponges (especially instructive in silica sponges), spongiously built bones, etc. An excellent example of systems with elements of microscopic cross-section is macerated skin (Fig. 59) or also latex tube systems of the latex plants. When algae threads are fished out of a pool we are astonished to find how they cling together in a tangled skein, although every thread is an individual in itself. Here the junction bonds, which are hypothetic in the case of gels, can actually be observed under the microscope, for at all points where two threads cross they stick together (compare Fig. 53b). The number of these junctions is so great that such a wad of algae is even, to a small extent, elastic when compressed.

We penetrate into the submicroscopic domain by making the threads so thin as to become invisible under the ordinary microscope, thus obtaining *gels*. Until recently their structural principles had to be found out by indirect means (FREY-WYSSLING, 1938). Nowadays, however, the reticular structure of gels can be imaged directly in the electron microscope (Fig. 60). As will be explained in the next paragraph, the submicroscopic strands or strings which form the gel frame will be designated as *micellar strings*. Thus the submicroscopic gel structure is a *micellar framework*.

The transition into the amicroscopic domain is of particular importance. Whereas

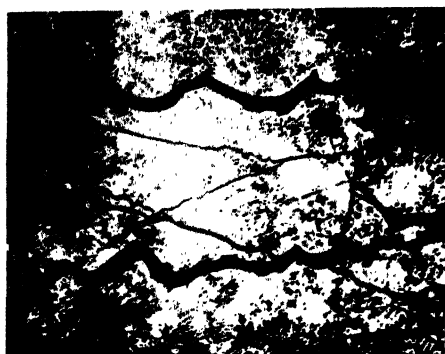



Fig. 57

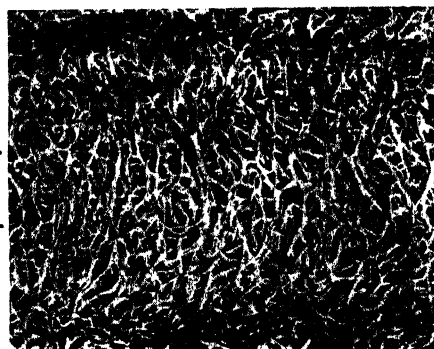



Fig. 58

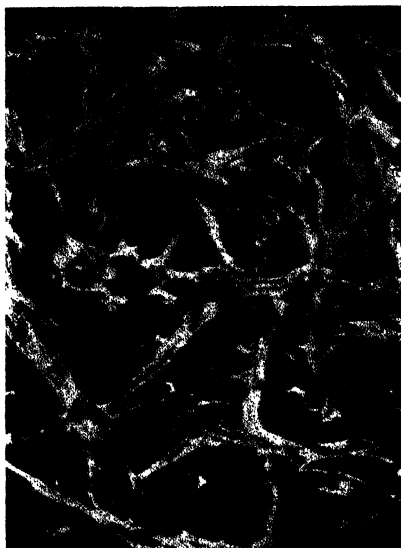



Fig. 59

Fig. 60

Reticular structures of different scales.

Fig. 57. Coarse macroscopic reticular structure: liane brush in a virgin forest photographed skyward. – Fig. 58. Macroscopic reticular structure: fascicular skeleton of a *Luffa* fruit (vegetable sponge). – Fig. 59. Microscopic reticular structure: network of collagene fibres in cow's skin (from KÜNTZEL, 1941). – Fig. 60. Submicroscopic reticular structure: ultra-structure of coagulated blood fibrine (from WOLPERS and RUSKA, 1939).

corpuscular disperse systems in this case become real solutions and are no longer accessible to colloid chemical methods of research, reticular systems remain colloids even if the thickness of the strands of their framework is reduced to amiscroscopic dimensions, i.e., to the cross-section of a single molecule. Thus in network systems

there is no lower limit to the colloid domain, they remain gels quite irrespective of whether their network is submicroscopic or amicroscopic. Such gels are, for instance, the polystyrene gels mentioned on p. 47 (Fig. 54), or the methyl cellulose gels prepared by TAVEL (1939) with the aid of oxalyl chloride, or pectin gels prepared by DEUEL (1947) with ethylene oxide. In these cases the strings of the network are *chain molecules* and the gel structure is a fine *molecular framework.*

Comparison of corpuscular and reticular systems. The properties of network gels differ in principle from those of sols with their corpuscular dispersed particles. This is clearly demonstrated by Table XI.

TABLE XI

COMPARISON OF CORPUSCULAR AND RETICULAR COLLOIDS IN THE SOLVATED STATE

		Corpuscular colloids	Intermediate state	Reticular colloids
Properties	Colloid state	Sols	Gel-solution	Gels
	Colloid portion	Individual particles (micelles or macromolecules)	Particles are impeding each other's motion	Coherent structure (micellar or macromolecular framework)
	Solvating liquid	Dispersing medium		Imbibition medium
	Equilibrium liquid in coacervation	Dispersing medium + colloid portion		Imbibition medium free from colloid
	Elasticity	Unelastic	Structural viscosity	Elastic
	Structure	Structureless	Beginning structure	Possessing a structure
Methods of research	Ultramicroscope	Demonstration of particles		Gel frame is optically empty
	Ultracentrifuge	Sedimentation		Syneresis
	Ultrafiltration	Particle size		Pore size
	Dialysis	With the aid of membranes		Without membrane
	Donnan equilibrium			
	Osmotic laws	Hold good	Disturbed	Do not apply, because gel is insoluble
	Kinetic migration	Mutual diffusion		Permeation
	Electric migration	Electrophoresis		Electrosmosis
Disturbance of equilibrium	Dilution, swelling	Unlimited dilution	Unlimited swelling	Limited swelling
	Disturbance of stability	Precipitation (flocculation, coagulation)		"Hardening" (tannage, fixation)
	De-mixing	Two coexisting "phases"		Usually vacuolisation

Whereas a liquid capable of solvatising a colloid substance will disperse corpuscular colloids, reticular colloids remain a coherent mass into which the solvating medium can only penetrate to a certain extent (limited swelling). Although in this case one also calls this a dispersing medium, because the colloid system is usually diluted, it would be better characterized as imbibition medium (see p. 57, 59), since the colloid substance is not dispersed into separate particles. In the coacervation of sols an

equilibrium liquid poor in colloid and the coacervate layer rich in colloid are formed (Fig. 21). In reticular coacervates, however, the equilibrium liquid contains no colloid because the latter is insoluble in the reticular state. For example, in the separation of gelatin from a gelatin solution no gelatin is found in the supernatant liquid (compare p. 17).

In reticular colloids the mutual position of the structural units is fixed; that is, a structure exists. It follows that gels possess a certain elasticity, although often a small one in those cases where the forces acting in the junction bonds forming the structure are weak. Typically intermediate between gels and sols are gel-solutions, whose particles impede each other's free movements. These gel-solutions, therefore, show structural viscosity, indicating the existence of a structure when deformed.

The difference between corpuscular and reticular systems is particularly apparent when testing the applicability of the methods of research developed by colloid chemistry. As a result of the close packing of the micellar strands all gels are optically empty in the ultramicroscope. In the centrifuge no definite sedimentation equilibrium is established; the imbibing medium is simply partly pressed out of the gel (syneresis). As the gels do not contain individual particles, ultrafiltration cannot be used as a method to discover whether they contain submicroscopic or amicroscopic structural elements. It gives some information, however, about the approximate pore size of the network structure, since on account of its structure each reticular colloid represents an ultrafilter, provided it possesses the firmness needed to resist the filtration pressures applied. In all further methods of research mentioned in Table XI the contrast between the movable particles of sols and the immovable frame of the gels finds expression. In dialysis and in the study of DONNAN equilibria, amicroscopic particles are removed by diffusion through a membrane which is impermeable to colloid particles. In the case of gels no membrane is needed, because its colloid portion itself is immovable and insoluble (see p. 127). For the same reason, the osmotic laws are not applicable to gels, whereas in true sols, where the individual particles are completely independent, they allow of a determination of the number (and therefore the weight) of the particles. Finally, when concentration gradients or potential gradients are applied to gels, the amicroscopic particles diffuse through the gel frame (permeation), or the imbibition liquid migrates through the electrically charged network (electrosmosis).

Similarly when the equilibrium in a colloid system is disturbed the behaviour of gels and sols is fundamentally different. Sols can be diluted by the solvating liquid, whereas in true gels only limited swelling occurs. In sols the disturbance of stability factors (hydration and charge) can lead to flocculation or coagulation. In contrast to what is commonly asserted, gels do *not* coagulate, they are "hardened". In technology this is denoted by tannage and in cytology by *fixation*. De-mixing of sols results in two microscopically uniform "phases" (Fig. 15, 21), whereas in gels the separated drops usually cannot unite and give rise to vacuolisation in the originally microscopically uniform system (Fig. 23). The concepts limited swelling, fixation and vacuolisation which are mentioned at the bottom of the last column in Table XI are familiar to all cytologists and we need not waste another word on the question which colloids are of the first importance in microscopic and submicroscopic morphology.

Indeed, the number of colloid systems in biology, whose nature has been

ascertained successfully by means of the methods of research developed for corpuscular dispersoids is very small (blood, milk, serum, suspensions of micro-organisms and viruses). No conclusive information could be derived by these methods on the fine-structure of the protoplasm. Even the very terminology of the theory of dispersoids which assumes dispersed particles in a dispersing medium is unsuitable. True, the introduction of *difform*, i.e., strongly anisodiametric particles, accounts to a certain extent for the properties known to modern macromolecular chemistry (MANEGOLD, 1941). The older technical terms of NÄGELI, however, (NÄGELI and SCHWENDENER, 1877) are much better adapted to the needs of biologists working with gels. NÄGELI's ideas can be applied to our present concept of gel structure. To that end let us first give a precise definition of the micellar concept, to which unfortunately various meanings have been attached in colloid science.

d. *Micellar Theory*

The concept micelle. C. NÄGELI was the first to develop a well-founded theory on the structure of hydrogels, which he designated as *organized substances* (new edition 1928). Starting from double refraction, anisotropy of swelling and layer structure of grains of starch (1858) and of cell membranes he made the assumption that these substances consist of long-shaped, submicroscopic particles with a *supermolecular* character and a *crystalline* structure. Such a particle was called a *micelle* (diminutive of the latin mica = crump).

Later, NÄGELI has extended his theory to solutions. According to him, when a gel is dissolved the micelles are maintained as units and give a micellar solution. As a result of this transference of the micellar concept from solid gels to solutions, this concept is used in the literature in various meanings. This was pointed out by several authors: ZSIGMONDY (1921), AMBRONN and FREY (1926, p. 152). Whereas the biologists, in particular AMBRONN's school (FREY, 1926a, 1928a) and also SCHMIDT (1934a) have sworn allegiance to the original definition which indicates the *form* and *crystallinity* of the particles, the meaning attached to micelles by colloid chemists is as a rule simply that of dispersed particles in a colloid solution, stressing in particular their *electrical charge*, without heeding their form and structure. In the latter case, therefore, it represents an overall concept which may embrace all possibilities such as primary particles (monones), secondary particles (polyones), associates (e.g., in soaps), etc., including their charges and solvation layers. As a result of this situation, the origin of this term is scarcely known in colloid chemistry. This led to what NÄGELI objected to in a discussion of PFEFFER's terminology in the famous "Osmotische Untersuchungen" (1877). NÄGELI says (new edit. 1928, p. 70/71): "PFEFFER uses the general expression 'tagma' for molecular compound, observing that in chemistry one would hesitate to introduce the term micelle, which is reminiscent of cell. It seems, therefore, that the etymological error is made: to believe that we are dealing with a barbaric composition of "cellula" and an unknown word beginning with "mi", in much the same way as the word aldehyde is formed".

The existence of long, submicroscopic particles in gels such as celloidin, denitrated cellulose, celluloid, gelatin, aluminium oxide fibres, etc. has been demonstrated unambiguously by AMBRONN by optical means (see p. 58). These particles often showed an intrinsic double refraction which could only be explained by assuming crystalline particles (AMBRONN, 1916/17). The existence of crystalline micelles in chitin (MÖHRING, 1922), in muscle fibres (STÜBEL, 1923) and in vegetable cell walls (FREY, 1926b) was demonstrated by means of the same methods.

At about the same time the crystalline nature of many colloid particles, for example gold sols, cellulose and many other colloids was established by the X-ray method (SCHERRER, 1920). NÄGELI's micellar theory was taken up by MEYER and MARK (1930) and propagated by them among chemists in almost unaltered form, after having been nursed for a long time in its original form by a few biologists. This is obvious from a comparison of NÄGELI and SCHWENDENER's scheme (1877) and the model of fibre structure given by SEIFRIZ (1929) and K. H. MEYER (1930): Fig. 61b. In NÄGELI's scheme (Fig. 61a) two intermicellar substances are drawn between the micelles; one of these substances may be eliminated. What is new in Fig. 61b is the determination of the *inner structure* of the micelles; for the rest, however, there is complete agreement with Fig. 61a. The micelles were considered as disperse phase, surrounded by intermicellar spaces which are accessible to the dispersing medium. To account for the coherence of the crystalline micelles in a solid framework, special micellar forces had to be assumed. MEYER and MARK considered these to be large cohesive forces which, in cellulose

for instance, are additively composed of the molar cohesions of the numerous OH-groups. However, since these same forces act intramicellarly as lattice forces, it was difficult to see what the difference might be between the forces responsible for the *intra*micellar coherence of the chain molecules in a crystal lattice and the *inter*micellar "micellar forces".

According to Nägeli, when a gel is dissolved, the micelles are dispersed, and the sol contains independent crystals. This viewpoint has often been adopted by others, in particular for cellulose sols, although such solutions do not give X-ray diagrams (for example Herzog, 1927). According

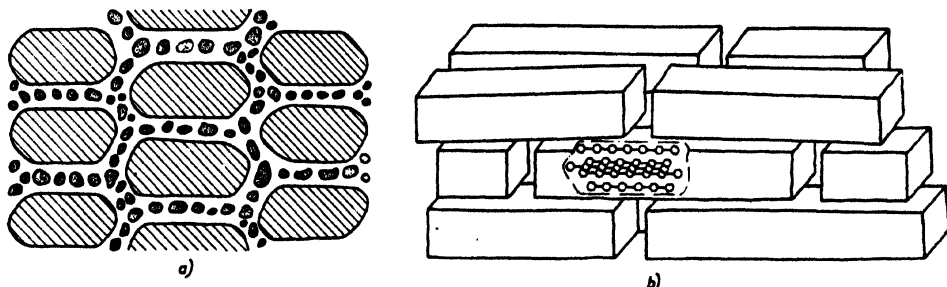



Fig. 61. Former conception of the micellar structure: *a*) according to Nägeli and Schwendener (1877), *b*) according to Seifriz (1929) and K. H. Meyer (1930).

to Staudinger (1932) the high polymer natural substances are dissolved as separate chain molecules instead of crystalline particles. At present therefore only crystalline suspensoid colloids such as gold-, vanadium pentoxide-, ferric oxide sols, etc. can be claimed to be micellar solutions in Nägeli's sense; they show mostly a strong birefringence of flow and partly also X-ray interferences.

In the case of gels further objections to Nägeli's concept of micelle are brought forward by our recent knowledge of the structure of high polymers. For, it is found that the chain molecules are much longer than the crystalline regions (Fig. 56). It follows that the micelles, instead of possessing individual character as assumed by Nägeli have grown together and are to a certain extent absorbed in the gel structure. Nowadays they can no longer be considered to be substantial (not even conditionally substantial) particles (Frey–Wyssling, 1936a, c; Kratky and Mark, 1937). They consist of well ordered chain molecules, which protrude from the crystalline into the amorphous regions and perhaps take part again in other ordered lattice regions.

We must conclude, therefore, 1. that the sols of physiological importance contain chain molecules which are more or less independent, rather than micelles in Nägeli's sense, and 2. that the micelles in gels do not represent independent crystallites but at best can be described as *lattice regions*. Taking into account the constant danger of confusion with the colloid chemical concept, which by micelle means an electrically charged instead of a crystalline particle, it would perhaps be better in our considerations to give up the concept micelle. If one wishes to use it nevertheless, one should not assign any special significance to this concept but simply use it in the sense of supermolecular colloid particle. This would exclude all possibility of confusion. One would then have to distinguish between two different kinds of colloid particles: 1. *super-molecular* micelles consisting of many molecules, and 2. *macromolecular* molecules of submicroscopic dimensions. However, since a well-founded terminology for sols does already exist, the micellar theory will be confined to gels, as originally intended by Nägeli.

Nomenclature. Although the assumption of independent micelles in gels has proved to be incorrect, Nägeli's work contains a great many other ideas on the structure of gels, which have been shown to be quite correct. I mention, for instance, the following paragraph (new edition 1928, p. 76/77): "Die Micelle vereinigen sich ... zu Verbänden ..., indem sie sich beliebig, bald baumartig, bald mehr netzartig aneinander hängen. Diese unregelmässigen Verbände ... bilden eine stehende Gallerte". Elsewhere he speaks of "Micellar-Reihen, in denen die Micelle miteinander verwachsen sind". Although at the time the existence of chain molecules was not even suspected, he has given a characterization of gel structure which is essentially correct.

Nowadays it does not matter so much in biology whether or not the living matter and its derivatives contain crystalline regions, but rather whether the particles are independent of each other as presumed in classical colloid chemistry or in the theory of dispersions, or whether they are united in a framework (however weak), and thus provide the colloid with a *structure*. Consequently, in contrast with structureless dispersoids, gels are in need of an appropriate terminology.

It is tempting to make up for this deficiency by creating new names[1]. However, one does not always render science a service by doing so, and it is perhaps more recommendable in this case to use old well-tried expressions adapted to modern experimental results by new definitions. Following NÄGELI, the frame substance will be designated as *micellar portion* and the intersticial substance as *intermicellar portion* of a gel. In those cases where the micellar structure consists of coarse beams or joists, which are partly crystalline and therefore homogeneous, one can also speak of *micellar phase* and *intermicellar phase*.

There is no danger that this new definition will again give rise to confusion, for the concept *intermicellar* is used in exactly the same sense as hitherto in the literature of the subject, and the concept *micellar* is only changed so as not to apply exclusively to the crystalline regions of a framework but to the framework as a whole. This solves the difficulty that gels whose framework units consist of only a few parallel chain molecules do not answer NÄGELI's original definition, because a small number of chain molecules are not capable of forming a crystal lattice. With still finer strands of the gel structure, it is true, it finally consists exclusively of chain molecules, and the micellar framework has changed into a *molecular framework*, as has been pointed out on page 52. Just as in the transition from colloidal to molecular dispersions there also exist transitional forms between (a) gels with micellar strands and (b) gels with chain molecules as structural units. In gels with a molecular framework the particle sizes of the two components of the system are not similar as in the case of solvent and solute molecules in a true solution. In principle they remain different in a morphological sense as framework and *interstitial substance*.

In the case of micellar systems possessing strands with a thickness of several molecules a difference should be made between processes which occur in the meshes of the network (*intermicellar*) and those occurring inside the beams of the frame, i.e., in the crystal lattice (*intramicellar*). In the same sense the expression "intramicellar" is used for cation exchange inside layer lattices (WIEGNER, 1935; BOTTINI, 1937). With the aid of the concepts "micellar", "intermicellar" and "intramicellar" all processes occurring in gel structures can be described unambiguously. By a relatively slight change in concepts we thus preserve a nomenclature which has done good service for 90 years, and renders honour to NÄGELI, who laid the foundations of the research on biological gels.

In Table XI we have recapitulated the most important points which according to our definition distinguish the reticular gel from its counterpart, the corpuscular sol. As in the case of dispersoids (Table II), the components of a gel can occur as gases, liquids or solids, with the restriction, however, that the micellar component must always be solid (Table XII). If the intermicellar substance is a gas or a liquid, we have to deal with network structures or capillary structures. If it is a solid, however, we have to deal with solidified gels, showing clearly in contrast to dispersoids

[1] PFEIFFER (1941b, 1942a) designates the theory of fine-structure as leptonics and the invisible structural units as leptones (from λεπτός = fine, small).

that the two components are completely *equivalent* as regards the arrangement in space.

TABLE XII
(Compare Table II)
RETICULAR SYSTEMS (ACCORDING TO FREY-WYSSLING, 1937d)

Imbibition medium	Micellar frame	Structures
Solid	Solid	Composite solid
Liquid	Solid	Gel structure
Gas	Solid	Capillary structure

The micellar structure is determined by the micellar strands, by the type of bonds between them and by the intermicellar substance. For a given type of micellar units, however, the gels can be built up with various different possibilities of orientation. This determines the *micellar texture* which gives information about the arrangement of the structural elements in the gel, in contrast to the *micellar structure* which characterizes the fine-structure in general.

Definitions. To sum up, we give the following survey:

By *structure*[1] we mean the fixed mutual positions of the submicroscopic or amicroscopic morphological units, by *texture*, however, the special arrangement and distribution of such structural units.

corpuscular colloids = colloids with freely moving particles
reticular colloids = colloids with a gel frame
macromolecules = molecular colloid particles
micelles = supermolecular colloid particles, most often packets of chain molecules in parallel arrangement
molecular framework = amicroscopic structure of interwined *chain molecules*
micellar framework = submicroscopic structure of coherent *micellar strands*
interstitial substances = substances in the interstices of a molecular framework
intermicellar substances = substances in the interstices of a micellar framework
intermicellar processes = processes occurring between the strands of a gel frame
intramicellar processes = processes occurring inside the strands of a gel frame
micellar structure = fine-structure of gels in general
micellar texture = arrangement of the structural units in particular

§ 4. STUDIES IN GELS

The colloid chemical methods of investigation which have proved so successful in the elucidation of the nature of sols have only a limited applicability to gels (compare the discussion of Table XI). Gels must therefore be investigated by different means. Of these we shall only discuss those which are of special importance to the investigation of cytological objects, while others which are for instance of importance to the tech-

[1] Not only crystalline but also amorphous solid phases possess a structure. For, in amorphous glasses (BÜSSEM and WEYL, 1936) and also in isotropic gels the structural elements are bound together elastically in fixed mutual positions, notwithstanding the lack of order. We must therefore in principle attribute a structure to all solid states of matter.

;sting of gels will not be mentioned. For lack of space the methods of investig- 'ill not be treated in great detail; we shall only deal with the principles of these ls and the problems which they can solve.

a. *Polarisation Microscopy*

Theory of composite bodies. The texture of gels can be explored by optical means if two conditions are fulfilled. In the first place the strands of the framework must be separated from the intermicellar space by definite phase boundaries, and secondly they may not be oriented at random but must show a certain tendency to orientation in a given direction in space. WIENER (1912) has calculated theoretically the optical effects occurring in these systems. In this calculation one must assume strongly idealized textures with, for instance, parallel circular cylinders or parallel planes (Fig. 62a and b). Such aggregates meeting the mathematical requirements are designated as "composite bodies" (German: Mischkörper). As the structural units (cylinders or planes) are not grown together, they do not possess a micellar structure in our sense. One can imagine, however, that a gel is formed out of such an idealized composite body if the structural units are somehow anastomised with each other. This does not affect the general character of the optical effects, but it is obvious that quantitative calculations according to WIENER's formulae cannot give very accurate results for gels with a micellar structure, since the geometrical conditions for an accurate mathematical treatment of the problem are not satisfied.

The rods or planes of the composite body must be supposed to be optically isotropic. It then follows from theory that the behaviour of the composite body with

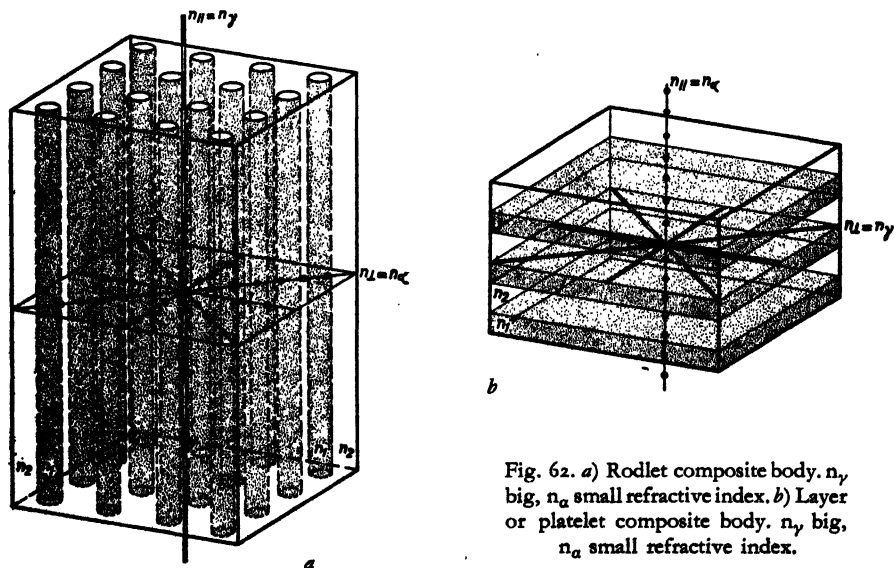



Fig. 62. *a*) Rodlet composite body. n_γ big, n_α small refractive index. *b*) Layer or platelet composite body. n_γ big, n_α small refractive index.

respect to light depends on the direction of its vibration, i.e., it is *anisotropic*, provided the diameter of the cylinders and the distances between the cylinders or layers are small compared with the wavelength of the light. It should be borne in mind that by "small" we do not mean arbitrarily small, as the structural units should possess true phase boundaries. Single chain molecules, for instance, cannot act as structural units in a composite body.

Optical anisotropy can manifest itself in three different ways:

1. Double refraction. The refractive power ($n_{//}$) for directions parallel to the axis of the composite body is different from that perpendicular to it ($n_\perp$) (Fig. 62a, b) so that, in polarised light interference colours occur as in double refracting crystals.

2. Anisotropic absorption (dichroism). In coloured composite bodies absorption is different parallel ($k_{//}$) and perpendicular ($k_\perp$) to the axis; they show therefore different

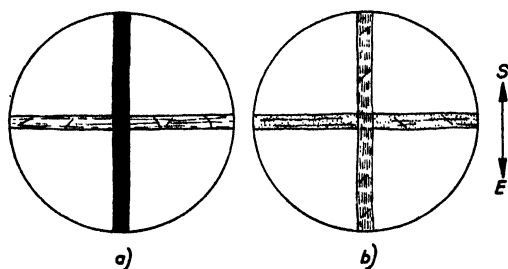



Fig. 63

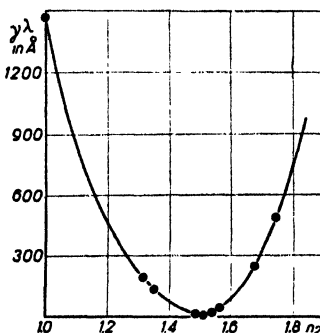



Fig. 64

Fig. 63. Dichroism of bast fibres (SE vibration plane of polariser) from FREY (1927b). *a*) Stained with chlorozinc-iodine: black/colourless, *b*) stained with gold: green (marked with little lines)/claret (dotted). – Fig. 64. Curve of rodlet birefringence of the epidermic hairs of incinerated barley awns (from FREY 1926b). Abscissa: refractive index n_2 of the imbibition liquid. Ordinate: retardation $\gamma\lambda$ in Å units.

colours dependent on their position with respect to the plane of oscillation of linearly polarised light (Fig. 63).

3. Anisotropic diffraction. Transmitted light is differently diffracted in different directions; the typical gloss of silk, for instance, must be attributed to this effect.

The composite bodies possess a very typical characteristic: ***their anisotropy is not constant*** but is a function of the properties of the substance enclosed between the particles, which in microscopy we designate as ***mounting*** liquid or better as ***imbibition*** liquid. The double refraction changes therefore with varying refractive power n of the mounting liquid. For this reason the double refraction of such composite bodies differs essentially from the double refraction of crystals, which represents a constant characteristic of the crystal.

Fig. 64 shows the changes in double refraction observed when epidermic hairs of incinerated barley awns are mounted successively in air (n = 1.00), water (n = 1.33), alcohol (n = 1.36), xylene (n = 1.49), benzene (n = 1.50), Canada balsam (n = 1.54), mono bromo naphtalene (n = 1.66), potassium mercury iodide (n = 1.72). The course of the birefringence in dependence on the refractive index n_2 of the imbibition liquid obeys a hyperbolic law. The double refraction is zero when $n_1 = n_2$ (n_1 = refractive index of micellar component). Composite bodies with rodlet texture are optically positive, those with layer texture are negative. WIENER's formula for the rodlet birefringence runs:

$$n_{//}^2 - n_\perp^2 = \frac{\delta_1 \delta_2 (n_1^2 - n_2^2)^2}{(\delta_1 + 1)\, n_2^2 + \delta_2\, n_1^2}$$

Here $n_{//}$ represents the extraordinary refractive index (parallel to the axis of the composite body) and $n_{\perp}$ the ordinary index (perpendicular to the axis), n_1 the refractive index of the isotropic rods and n_2 that of the imbibition liquid; δ_1 and δ_2 are the volume fractions of the two components ($\delta_1 + \delta_2 = 1$). Clearly, $n_{//}^2 - n_{\perp}^2$ is a measure for the double refraction $n_{//} - n_{\perp}$. The formula shows how this double refraction depends on the refractive index n_2 of the imbibition medium. It is zero when $n_1 = n_2$, and positive for all other values of n_2, because the numerator contains the square of $n_1 - n_2$. In other words, the rodlet birefringence is always positive: $n_{//} > n_{\perp}$. Since in birefringent objects the larger index is denoted by n_γ and the smaller one by n_α, it follows that $n_{//} = n_\gamma$ and $n_{\perp} = n_\alpha$. Conversily, in composite bodies with layer texture and negative birefringence we have $n_{\perp} = n_\gamma$ and $n_{//} = n_\alpha$.

It is important that besides the volume fractions δ_1 and δ_2 no quantities depending on the dimensions of the rods occur in the equation. The double refraction is independent of the thickness of the rods. This is of great importance to the study of submicroscopic textures, because in this region no measurements of the thickness can be performed.

The double refraction of the composite bodies may be designated as *textural birefringence*[1], because its nature depends on the texture of the solid phase. The curves of textural birefringence are therefore used to examine whether intermicellar spaces occur in a material, and to decide whether the micellar phase has the form of rods or platelets. Usually one does not measure the double refraction $n_{//} - n_{\perp}$ itself, since it depends on the variable thickness d of the swollen gel according to the formula

$$n_{//} - n_{\perp} = \gamma\lambda/d.$$

Rather one simply measures the retardation $\gamma\lambda$, where γ is the so-called phase difference and λ the wavelength of the light. The introduction of this method of research into colloid optics is due to AMBRONN.

Measurement of the double refraction. The basic formula for double refraction can be simplified by introducing the notations Δn for $n_{//} - n_{\perp}$ and Γ for the retardation or path difference $\gamma\lambda$. This gives

$$\Delta n = \Gamma/d,$$

which shows clearly the linear dependence of the retardation on the thickness d of the object, because Δn for a given object in a given medium is constant.

The formula applies to objects in the form of discs bounded by two parallel planes as, for instance, in microtome sections, where d corresponds to the thickness of the section. Many biological objects, however, (myelin worms, myelin sheath of the nerves, fibres with narrow lumen, etc.) occur in the form of hollow cylinders. In this case the thickness increases with increasing distance from the edge, and accordingly the path difference increases. The phenomena are particularly complicated when the optical axis is not parallel to the axis of the cylinders as in fibres, but perpendicular to the cylinder axis, as is the case of myelin objects. The birefrin-

[1] In German I have termed this phenomenon as "Form-Doppelbrechung" (FREY, 1924), with reference to WIENER's theory, where a "form coefficient" is used to characterize the shape of the submicroscopic units. The translation of this term is difficult, as "form birefringence" is impossible and "birefringence of shape" (F. O. SCHMITT) or "birefringence of form" (RUNNSTRÖM) are not very obvious either. "Structural birefringence" is no equivalent, as the intrinsic double refraction of crystals is caused by their structure. I, therefore, propose the term "*textural birefringence*", which is a clear and self-evident expression.

gence Δn may then be calculated from a formula of BEAR and SCHMITT (1936) if the largest possible path difference $\Gamma_{(max)}$ is measured. This formula runs:

$$\Delta n = \frac{3\,\Gamma_{(max)}}{(d_1 + 2d_2) \arccos [(d_1 + 2d_2)/3d_1]}$$

where d_1 represents the diameter and d_2 the inner diameter of the hollow cylinder.

A similar problem occurs in the determination of the double refraction of objects with spherite structure and radially oriented optical axis (e.g., grains of starch). In this case the double refraction is

$$\Delta n = \frac{\Gamma_{(max)}}{1.122\,r}$$

where r is the radius of the spherites (FREY-WYSSLING, 1940b). The formula of BEAR and SCHMITT should yield this formula for a solid cylinder, where $d_2 = 0$. This is not the case, because empirical data concerning the position of the maximum retardation $\Gamma_{(max)}$ in a myelin tube have been mixed up with the optical theory (SCHMITT and BEAR, 1937).

Sign of the double refraction. It is very important for the textural analysis of gels to know the sign or character of the double refraction. A micellar texture is called optically positive if, as mentioned before, $n_{//} - n_{\perp}$ has a positive value. If, on the other hand, $n_{//} - n_{\perp}$ is smaller than zero, the double refraction is negative. The refractive index $n_{//}$ always refers to a direction which in some way or other is of a special character: direction of the orientation in the composite bodies mentioned, direction of growth, direction of pressure or tension, direction of flow, special morphological direction, and so on. In fibres and threads, for example, the fibre axis is the reference axis, in cross-sections of parenchym cells the tangential direction, in spherites the radial direction.

The character of the birefringence of gels is indicated by the so-called index ellipsoid, the long axis of which corresponds to the larger index n_γ, while the short

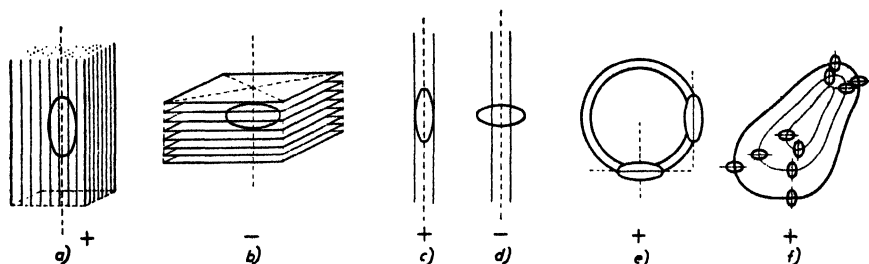



Fig. 65. Optical character of gels. Reference axis marked by a dotted line. *a*) Rodlet composite body, *b*) layer composite body, *c*) thread of gum arabic, *d*) thread of cherry gum, *e*) section across a vegetable parenchyma cell (reference axis = tangential direction), *f*) starch grain (reference axis = radial direction).

axis corresponds to the smaller index n_α. The direction of n_γ is determined by comparison with a selenite platelet (see AMBRONN and FREY). The orientation of the index ellipsoid and the direction to which the double refraction refers has been drawn in Fig. 65. Many gels are isotropic when observed in the direction of the reference axis;

they are uni-axial in the crystallographic sense, and the definition of optically positive and negative is in complete conformity with the terminology customary in mineralogy. In those cases, however, where the object shows anisotropic behaviour towards light incident along the reference axis, crystal optics use other definitions to describe the optical character, and the customary terminology in gels is no longer identical with that in crystal optics. Whenever there exists a direction of isotropy, this should be chosen as reference axis.

Systematics of double refraction. In most cases the micellar texture itself is birefringent, because the chain molecules constituting the strands of the structure are themselves anisotropic. This kind of optical anisotropy is called *intrinsic double refraction.* In this case the double refraction of the gel cannot be reduced to zero by changing the refractive index n_2 of the imbibition liquid; there is a residual double refraction in the minimum of the curve for textural birefringence: the intrinsic double refraction of the substance. In all cases examined so far, the micellar strands behave like optically uni-axial systems, or at least so to a first approximation. They possess, therefore, two principal refractive indices, designated by n_e (extraordinary index along the fibre axis) and n_o (ordinary index perpendicular to the fibre axis). The intrinsic double refraction is accordingly $n_e - n_o$. As a rule it is positive, but sometimes turns out to be negative. In those cases where the intrinsic double refraction is different from zero, the refractive index n_1 in WIENER's formula is to be replaced by the average value $\frac{1}{2}(n_e + n_o)$ or even better by $\frac{1}{3}(n_e + 2n_o)$.

Both types of textural birefringence (positive composite bodies with rodlet texture and negative composite bodies with layer texture) may be combined with the three possibilities: positive, negative and zero intrinsic double refraction. Upon the whole one can, therefore, distinguish between six types of double refraction (FREY, 1924).

Both the textural and the intrinsic birefringence can be attributed to the structure of the object, but the intrinsic double refraction is caused by the much finer structure of the crystal lattice, whereas the textural birefringence results from the coarser colloid structure. The latter is, therefore, as a rule smaller than the former.

The intrinsic and the textural double refraction are both due to *morphological properties*, in contrast to the phenomenon of *incidental double refraction*, which becomes apparent when solid objects are subject to tensions or pressures, and which therefore are also designated as *double refraction due to tension* or *tension double refraction.* This phenomenon accompanies the elastic deformation (photo-elastic effect), and elastic deformability is a condition for its occurrence. Since, according to definition, gels actually do possess this property (Table XI), effects of this kind are to be expected in gels exposed to tensions. The tension double refraction is usually positive with respect to the axis of deformation, while that due to pressure is usually negative. The effect is most pronounced in isotropic gels (e.g., gelatin plates moulded tension-free), but is of course also observed in gels which are anisotropic by nature if these are exposed to tensions, in which case it is superposed on the textural and intrinsic double refraction existing beforehand. On removal of the tension, the tension double refraction must disappear, as every really elastic phenomenon. If it does not, the object has been plastically deformed. The photo-elastic effect is due to the deformation of electron orbits in the material concerned; the distances between the atoms in this material are slightly increased or decreased. In cubic crystal lattices these changes in atomic distances have been the subject of a careful study (for instance WIENER, 1926b).

Orientation double refraction. Since the junction bonds in a gel are seldom very strong, they easily yield to the forces applied. The elastic deformation is then followed by a re-orientation of the micellar strands, thus intensifying the intrinsic and textural birefringence of the gel. For this reason the optical phenomena in gels exposed to mechanical action are often very complicated. The difference between the double refraction due to tension and that due to orientation is most obvious when these phenomena are different in sign, as for example in the basic experiments of AMBRONN (1889) with cherry gum. For, when stretching cherry gum, the transient, weakly positive double refraction resulting from the tension is followed by a negative double refraction due to the orientation of the micellar texture.

Distribution of orientations. In a stretched gel the directions of the micellar units are spread about the reference axis according to a complicated distribution function (KRATKY, 1933, 1938). The majority of micellar strands enclose small angles with the direction of the stretch, and only few of them enclose large angles with this direction. The distribution function depends on the degree of stretch. If the degree of stretch is unknown, however, an idealized scheme of the distribution can be made by assuming that within a certain angle all possible orientations about the reference axis occur with equall frequency. The assembly of orientations then forms a sector (in a plane) or a cone (in space), whose vertical angle α can be computed from the double refraction of the gel when the intrinsic double refraction $n_e - n_o$ of the micellar strands is known, provided one takes care to choose the imbibition liquid in such a way that textural birefringence is negligible. The angle of scattering α is then given by the following simple relation (FREY-WYSSLING, 1943a):

$$\text{for scattering in a plane } \Delta n = (n_e - n_o)\frac{\sin 2\alpha}{2\alpha}$$

$$\text{for scattering in space } \Delta n = (n_e - n_o)\frac{\cos\alpha + \cos^2\alpha}{2}$$

For example, the space angle in cellophane paper, referred to the preference direction, imposed by the manufacturing process, was found to be 71°.5. The anisotropy of cellophane is, therefore, rather strong, for the angle of scattering corresponding to the isotropic state, i.e., completely uniform distribution, would have been 90°. The micellar strands with their numerous orientations in space may be replaced by a gel in which only a single orientation occurs. This orientation angle is called the average orientation angle α_m. With the assumptions made by us α_m becomes $\frac{1}{2}\alpha$, as shown by Fig. 66a.

The orientation of the strands in a micellar texture can be brought about by a variety of means other than tension or pressure, for example by drying or freezing a gel (AMBRONN, 1891; ULLRICH, 1941); the strings or strands of the frame are then shoven more or less into parallel positions.

Birefringence of flow. The best defined orientation however, is that in a field of flow, if one succeeds in liquifying the gel to a sol by releasing the junctions. If such a solution is subject to flow, the colloid rodlets are turned parallel at all points where a velocity gradient exists. A well-defined velocity gradient can be obtained by bringing the sol in a narrow gap (width below ½ mm) between a fixed hollow cylinder and a revolving inner cylinder (SIGNER, 1930, 1933; BOEHM, 1939; FREY-WYSSLING and WEBER, 1941). When rotating the inner cylinder, the liquid in contact with the surface of the rotor acquires its velocity, while the liquid in contact with

the wall of the fixed cylinder remains at rest. As shown by Fig. 66b this gives rise to a velocity gradient in the gap, and thus to a force couple on the rodlets dissolved. This force couple, however, is counteracted by the Brownian movement of the particles, which tends to annihilate the orientation brought about by the shear. As a result of this competition between orienting forces and Brownian movement the rodlets are scattered with respect to the axis of orientation. The distribution function of the rodlets is very complicated, but can be derived from theory (see, for instance,

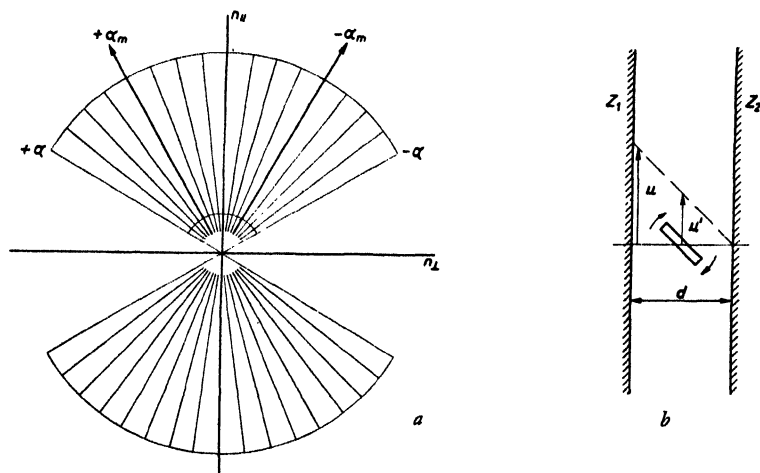



Fig. 66. *a*) Scattering of rodlets when orientated; *a* angle of scattering. *b*) Orientation of rodlets by a gradient of flow. z_1 revolving inner cylinder, z_2 immobile outer cylinder, d gab between the two cylinders, u maximum velocity of flow, u' velocity of the considered rodlet which is orientated by the velocity gradient.

PETERLIN and STUART, 1943). It is found that the direction of the axis of orientation depends on the length of the rods. With short rods (axis ratio a : b $\gtrsim$ 1) the orientations are spread about an axis enclosing an angle of 45° with the direction of flow. With increasing length of the rods (a : b $\gg$ 1), the axis tends to be orienttated in the direction of flow, finally (when a : b $\rightarrow \infty$) to become parallel to the tangent plane of the cylinder. The direction of the axis of orientation can be ascertained in the polarisation microscope as direction of extinction. The *extinction angle* allows therefore of conclusions on the length of the micelle rodlets or macromolecules dissolved, since short particles give extinction angles of about 45° whereas filaments give angles near 0°.

Having determined the extinction angle, one can also measure the retardation (technical notes in WISSLER, 1940, and historical review in PILNIK, 1946).

The birefringence of flow is no constant as is the double refraction of crystals, because the retardation does not only depend on the thickness of the layer, but also on the velocity gradient and the viscosity, and on the concentration of the solution. All these variable quantities are combined in MAXWELL's constant which enables one to characterize and to compare the anisotropy of flow of different sols. In those cases where the particles of the solute are chain molecules (molecular colloids), the

method can be used to obtain data on the anisotropy of single macromolecules.

In the case of single chain molecules we can no longer speak of refractive indices, since the surface of a molecule does not represent a phase boundary where the velocity of propagation of light is changed by a definite amount. The optical properties of the molecules are therefore characterized by another quantity, designated as *optical polarisability*, which is a measure for the influence of the electromagnetic field of a light wave on the orbits and oscillations of the electrons in the molecule. This influence depends on the direction of vibration of the light, and in a rod-shaped molecule with rotational symmetry we must therefore distinguish two different principal polarisabilities, the one parallel and the other perpendicular to the molecule axis, in the same way as we must distinguish two principal refractive indices in an optically uni-axial crystal.

More than once the question has arisen (for instance, SCHMIDT, 1938) whether chain molecules also cause rodlet birefringence as micellar strands do, when they are arranged in a parallel alignment. This problem has been solved by SADRON, (1937). It follows from the theory developed by him that the formula for the double refraction of flow consists of two parts. The first part depends only on the polarisability of the molecule (compare intrinsic double refraction), whereas the second part contains also the influence of the shape of the particles (compare textural birefringence). In contrast to the conditions prevailing in micellar systems, however, both terms depend on the refractive index of the solvent (FREY–WYSSLING, 1943b).

Micellar textures. Some examples will demonstrate the results obtained so far in the optical structure analysis of gels (FREY-WYSSLING, 1930). The majority of gels to be considered possesses a micellar framework containing regions of lattice order with rod-shaped crystals. In the following schemes these are indicated by dashes, although it should be remembered that these lattice regions do not represent isolated dispersed particles but that they are all interlinked and interwoven by chain molecules.

When it has been ascertained by a combination of optical results and X-ray analysis or birefringence of flow, that the rod-shaped lattice regions or the chain molecules are optically positive with respect to the longitudinal axis, the orientation of the lattice regions can be derived from the character of the double refraction in various sections of the gel. This can be demonstrated in particular in the case of all walls of anisodiametric plant cells. As shown in Fig. 67, the orientation of the lattice regions is indicated by the arrangement of index ellipsoids in radial, tangential and cross-section.

In the secondary wall of a bast fibre the lattice regions run almost parallel to the axis (*fibre texture*, Fig. 67a). If their orientations are scattered with respect to the cell axis, the cross-section which in the first case is almost isotropic becomes birefringent; we obtain a *fibroid texture* (Fig. 67b). The counterpart of the fibre texture is the *ring texture* (Fig. 67c) in which all lattice regions run in tangential orientation. This texture occurs in the ring-shaped reinforcements of young vascular cells and probably also in the primary membrane of fibres. If, starting from this texture which is optically negative with respect to the cell axis, the lattice regions are allowed to scatter, the wide-spread *tube texture* is obtained (sieve tubes, latex tubes, vessels, longstretched parenchym cells, etc.). Here the tangential section is optically negative; the radial section, however, is positive, since all projections of the scattered rod-shaped lattice regions upon the radial section are approximately parallel to the axis. As there is a continuous change from the negative region to the positive one, a front view of

these cells will show an isotropic zone in which the two regions of opposite sign become merged (Fig. 67d).

If the lattice regions do not scatter, but deviate from the direction of the cell axis while they remain parallel to each other, a *screw texture* is obtained, as is characteristic for cotton wool fibres, the tracheids of conifers (JACCARD and FREY, 1928; PRESTON, 1934, 1946) and the wood fibres of deciduous trees.

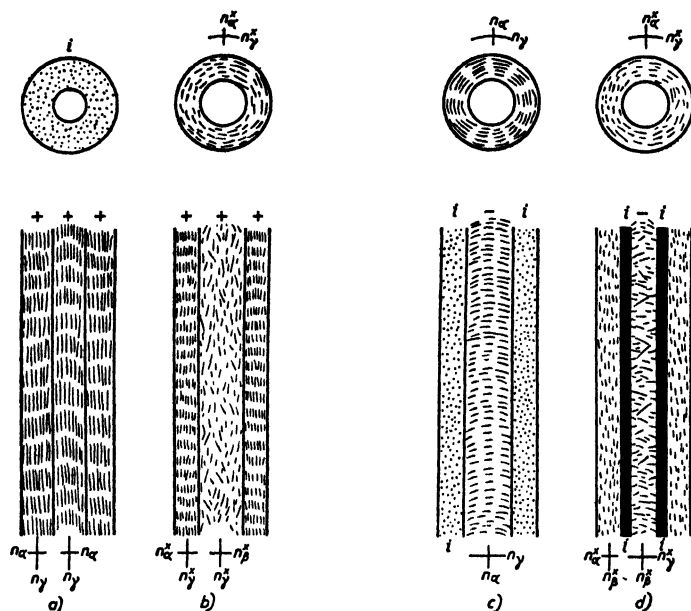



Fig. 67. Micellar textures of cell walls (from FREY-WYSSLING, 1930). *a*) Fibre texture, *b*) fibroid texture, *c*) ring texture, *d*) tube texture. n_γ biggest, n_α smallest refractive index of cellulose; n_γ^* biggest, n_β^* medium, n_α^* smallest refractive index of the cell wall. i isotropic, + optically positive, — optically negative.

In isodiametric objects there exists no morphological axis which may serve as reference axis to the double refraction. In spherical objects such as starch grains, spherites and the like, the radial direction is therefore chosen as reference axis. If the refractive power for vibrations parallel to this axis is larger than that for vibrations in tangential direction, the spherite texture is called positive, while negative in the opposite case. The determination of the optical character of a spherite built up of chain molecules or rod-shaped lattice regions, however, does not suffice to derive its submicroscopic texture. For, as shown in Fig. 68, spherites can be positive or negative both with radial and with tangential arrangement of the structural elements, dependent on whether the structural elements themselves are positive or negative with respect to their axis. In the first place one must, therefore, ascertain the optical character of these structural elements. In most cases one has to deal with the texture shown in Fig. 68a (starch grains, inulin).

In hollow spheres, the reference axis cannot be determined unambiguously. In isodiametric parenchym cells, for instance, the double refraction of the cell wall is eferred to the tangential direction (Fig. 65e), in analogy to the situation in aniso

diametric cells, although they are isotropic in radial direction. This is due to a at random orientation of the structural elements in the tangent plane. An arrangement of this kind is designated as *foliate texture*. For further details of optical texture analysis we must refer to the literature concerned (AMBRONN and FREY, 1926; FREY, 1926b; FREY-WYSSLING, 1930, 1935a; SCHMIDT 1934a, 1937a).

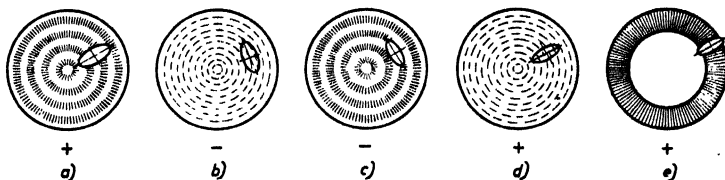



Fig. 68. Gels with spherite texture. *a*) Positive, *b*) negative spherite of positive rodlets; *c*) negative, *d*) positive spherite of negative rodlets; *e*) positive myelin sphere (oblate) of positive rodlets.

b. *X-ray Analysis of Gels*

Micellar strands. A complete structural analysis by means of X-rays is only possible if crystalline lattice regions are present. In the case of molecular colloids such as rubber solutions, protein solutions, etc., the irradiation with monochromatic X-rays furnishes as a rule no more than an "amorphous" ring, which gives some information about the *intra*molecular periods occurring most frequently (for instance, in rubber: the length of an isoprene unit). Only when the chain molecules are arranged in a crystal lattice does X-ray analysis yield interference phenomena rich in lines or spots, from which far-reaching morphological conclusions can be derived. This will be further illustrated by means of the cellulose diagram of ramie fibre (Fig. 69). Each point on the diagram corresponds to a set of parallel net planes in the crystal lattice. The diagram of Fig. 69 allows of measuring four quantities: 1. the mutual distances,

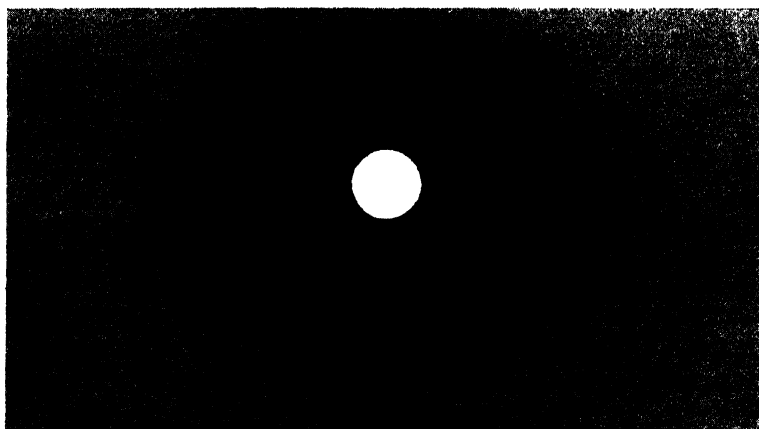



Fig. 69. X-ray fibre diagram from ramie showing layer lines.

2. the density, 3. the breadth and 4. the arrangement of interferences, each of which allows of calculating a corresponding quantity in the undisturbed lattice regions.

1. According to BRAGG's law of reflexion, the distance between the lattice planes is calculated from the distance between the interferences and the centre of the diagram. It follows from X-rays optics how the unit cell (see p. 20) in the crystal lattice of cellulose can be computed from the distances measured in the diagram of artificially orientated cellulose preparations whose crystalline regions display an arrangement of even higher orientation than in ramie fibres. The elementary cell found for crystalline cellulose is monoclinic; its sides are a = 8.35 Å, b = 10.3 Å, c = 7.9 Å, and the angle β between a and c is 84° (MEYER and MARK, 1930). Of these quantities, the fibre period b which corresponds to the length of a cellobiose molecule has been determined the most accurately (Fig. 70). It is calculated from the distances between the so-called layer lines which are clearly visible in Fig. 69, running parallel to the equator of the diagram and connecting, as it were, the interference spots. These interference spots are broadened along the layer lines as a result of cellulose chains which do not belong to the crystal lattice (SAUTER, 1937).

Fig. 70. Crystal lattice of cellulose (according to MEYER and MISCH, 1937).

2. From the *density of interferences* the number of atoms in the net planes can be derived, since the lattice planes reflect the X-rays more intensely the more atoms they contain. The density of interferences can be estimated, or measured photometrically. In Fig. 69 two black spots can be seen on the equator, with a mutual distance of 26½ mm. Their great density is caused by the family of net planes which contain the glucose rings of the cellulose chains and, since both points correspond to the front plane of Fig. 70, the ring of the glucose units must lie in this plane. In this way it is possible from the intensity of the interferences to determine the orientation of the molecular models (obtained on structural chemical grounds) in the unit cell (derived from X-ray analysis).

3. From the *breadth of the interferences* one can calculate the width of the undisturbed lattice regions, using a method developed by SCHERRER (1920) for metals, i.e., substances absorbing X-rays, and worked out by LAUE (1926) for non-absorbing substances. To this end the density must be measured photometrically. The breadth at half-maximum of the density peaks in the photometer curve (Fig. 72) is a measure for the dimension of the crystalline regions perpendicular to the set of net planes causing the interference. This dimension is the smaller the broader the X-ray interference in the diagram. In Fig. 69 the interference spots on the equator are clearly broader than those near the poles of the diagram. It follows from this, that in the fibre the dimensions of the lattice regions are considerably smaller in directions perpendicular to the fibre axis than in directions parallel to his axis. They must, therefore, be rod-shaped, in conformity with the conclusion drawn from the character of their textural birefringence. HENGSTENBERG and MARK (1928) find 50–60 Å for the thickness of these rodlets. Their length cannot be measured accurately, since the formulae are very insensitive to changes in length when this length is large (FREY-WYSSLING, 1937a). The experiments admit of no doubt, however, that the length of the rodlets

must be more than 10 times and probably even a great deal more than 10 times as long as their thickness.

4. From the *arrangement of interferences* the arrangement of the rod-shaped lattice regions can be derived. In the diagram considered all rodlets are parallel to the fibre axis (*fibre diagram*). If, however, they follow a screwline within the wall, the interferences on the equator are drawn out into sickles (*sickle diagram*). Finally, if they lack all order, interference rings instead of spots are obtained (DEBYE-SCHERRER or *ring diagram*, see Fig. 71). A comprehensive and simple treatment of the relations between the arrangement of interferences and that of lattice regions has been given elsewhere (FREY-WYSSLING, 1935a, p. 11). Ring, sickle and fibre diagrams are represented in Fig. 77.

Working out the fibre diagrams in all detail from the four points of view mentioned, one arrives at the structural model of the fibre wall shown in Fig. 61b. This picture renders all the facts which can be ascertained by means of X-rays, be it that the rodlets are in reality much thinner.

When drawing such a scheme it should always be borne in mind that X-ray analysis only gives information about the regions of lattice order; no information can be obtained in this way about the *regions without lattice structure*. In particular, it cannot be decided by means of X-rays whether the chain molecules in the crystal lattice are of exactly the same length as the lattice regions or whether (as has already been mentioned) they protrude from these regions in an unordered manner and take part in several other lattice regions (Fig. 56). X-ray analysis therefore cannot give any information about the manner in which the crystalline regions are interlinked or about the interstices between the regions of lattice order. From a biological point of view, however, these *intermicellar spaces* are of special importance. For, in most substances possessing a framework, the micellar strands with its crystalline regions should be considered to be practically lifeless, while all perceptible processes of life presumably take place in the intermicellar system. Thus, the mechanical properties of a gel are determined in the first place by the micellar structure, whereas for all physiological questions (such as permeability, metabolic processes, vital staining, etc.) one should study primarily the intermicellar regions.

Intermicellar spaces. The regions between the meshes of the micellar framework may represent a homogeneous phase if they are filled with a uniform liquid or gas. This only holds good so long as the gel frame consists of strands which can themselves be considered as a phase, so that a phase boundary exists. If the strands become so thin, however, as to reach the dimensions of a chain with the thickness of a single molecule, the concept phase loses, of course, its significance.

Informations about the dimensions of the intermicellar spaces in the gel frame can be obtained in various ways. If one succeeds in filtering particles of known size through a gel, one can conclude that the pores should be bigger than the particles, as in an ultrafilter. Unfortunately, however, it is not possible to obtain absolute values of the pore size of the intermicellar spaces with the aid of ultrafiltration (CZAJA, 1930), since differences in electric charge or in chemical behaviour (hydrophoby) very strongly influence the ease with which filtration of the particles takes place (MORTON, 1935). For this reason, only relative sizes can be obtained, which cannot be compared with the absolute values determined by means of X-rays.

Until now it has not been possible to obtain X-ray diagrams of the intermicellar substances. For even when in the solid state they usually do not show the properties of crystals. In the cell wall, for instance, silicic acid, lignin, cutin, etc. are embedded

in the amorphous state and therefore do not give rise to X-ray interferences. For this reason one was completely ignorant of the amount of space occupied by the intermicellar regions in frameworks. To obtain informations in this important field, foreign substances must be introduced into these spaces, where they crystallise and can then be submitted to X-ray analysis (FREY-WYSSLING, 1937a). We must therefore create by artificial means an intermicellar substance possessing lattice order, which

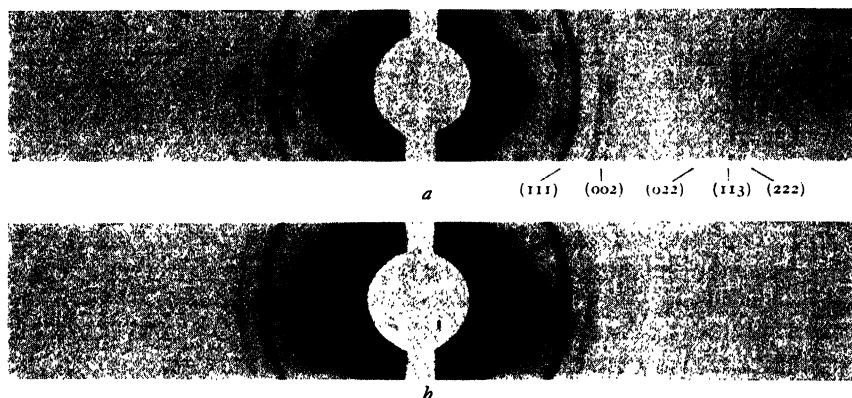



Fig. 71. X-ray diffraction pattern of *a*) ramie and *b*) silk stained with gold. In addition to the fibre diagram DEBYE-SCHERRER rings of gold (111), (002) etc. are seen (from FREY-WYSSLING, 1937a).

allows of deriving quantitative data about the dimensions of the unknown submicroscopic regions. To this purpose, gold and silver crystals have proved to be the most suitable. Following AMBRONN, the objects are soaked in 1–2% solutions of gold chloride or silver nitrate, then carefully dried with blotting paper, finally the salt absorbed is reduced by means of light or hydrazin hydrate (FREY, 1925b). In this way microscopically homogeneous colourings are obtained which show a beautiful dichroism (compare AMBRONN and FREY, 1926, table of colours). The X-ray diagram of the dyed fibres shows DEBYE-SCHERRER rings of crystalline silver or gold (Fig. 71) in addition to the fibre diagram of the framework substance (ramie fibre, silk and wool). The ring-shape of the interferences proves that the metal crystallites imbedded take up all possible positions with respect to the fibre axis. The size of the cubic gold and silver crystals is calculated from the breadth at half-maximum of the interference rings (Fig. 72).

The investigation led to the surprising result, that in silk and wool metal particles with a cross-section of about 50 Å and in ramie fibres even those with a cross-section of over 100 Å are incrusted (Table XIII). Since the strands of the micellar framework in ramie fibres have a thickness of only 50 Å, the artificially embedded metal crystallites occupy an unexpectedly large space. Notwithstanding their great strength, cellulose fibres must, therefore, be built rather loosely. Besides, this was already to be expected from density measurements in the bleached fibres used in these experiments. After removal of all substances incrusted, the density of ramie fibres amounts to only 1.39, whereas the density of cellulose is 1.59. There should therefore be about 12.6% of submicroscopic empty space[1] (FREY–WYSSLING and SPEICH, 1942).

[1] The density 1.39 ± 0.03 is derived from accurate determinations of mass and volume. If, instead of the density of crystalline cellulose, one uses the density 1.55 of the incompletely crystallised fibre measured in toluene, one finds a discrepancy of 10.3%.

TABLE XIII

PARTICLE SIZE Λ OF GOLD AND SILVER CRYSTALS INCRUSTED IN FIBRES

	Metal incrusted	Λ in Å
Ramie fibres	Ag	85
Ramie fibres	Au	84
Hemp fibres	Au	90
Bamboo fibres	Au	83
Wool	Au	58
Silk	Au	50

It is clear that not all cellulose rodlets with a cross-section of about 50 Å can be surrounded by spaces 100 Å wide, since otherwise the discrepancy in density should be much greater still. Furthermore, the phenomena of swelling require very narrow intermicellar spaces of the order of 10 Å, into which the water can penetrate, pushing the cellulose rodlets apart. In dyed ramie fibres there must therefore be two categories of submicroscopic spaces: 1. narrow intermicellar spaces of the order of magnitude 10 Å which are responsible for the phenomena of swelling, and 2. coarser capillary spaces which are accessible to dyes of much larger dimensions and to the so-called incrusting substances lignin, cutin, etc. For this reason they are of primary importance technically in the process of dyeing and physiologically in the incrustration of the cell wall. It must be supposed that these coarser spaces are widened by the growth of the substances embedded.

The capillary shape of the coarser spaces can be proved by the following consideration: in objects with a well-developed fibre texture the gold and silver particles

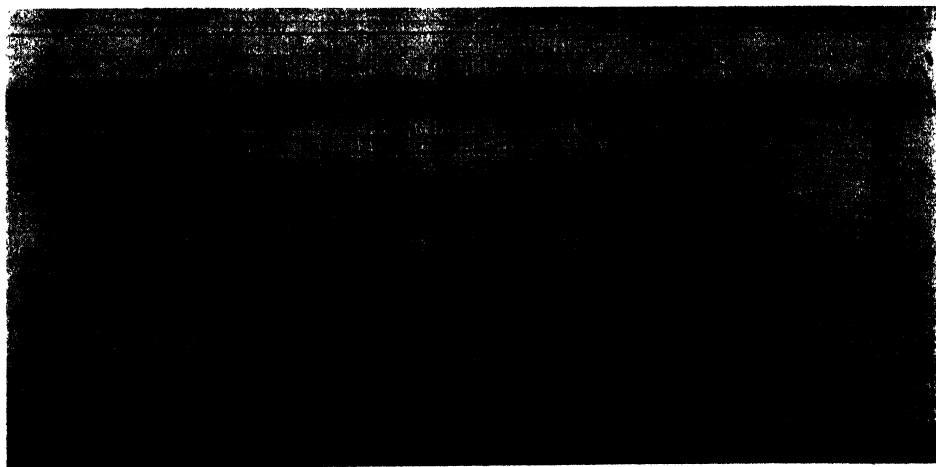



Fig. 72. Photometer curve of hemp fibres stained with gold (the distance and breath of the interferences are 2.0 times magnified as compared with Fig. 71). The breadth at half-maximum of the density peaks allows of measuring the diameter Λ of the embedded gold crystals (from FREY-WYSSLING, 1937a).

embedded give rise to a strong rodlet dichroism (FREY-WYSSLING and WÄLCHLI, 1946). This is only possible if the isodiametric metal crystals are arranged in rows or

in rod-shaped aggregates; i.e., the metal particles must lie in pre-formed submicroscopic canals. Still further conclusions can be drawn from experiments with silver

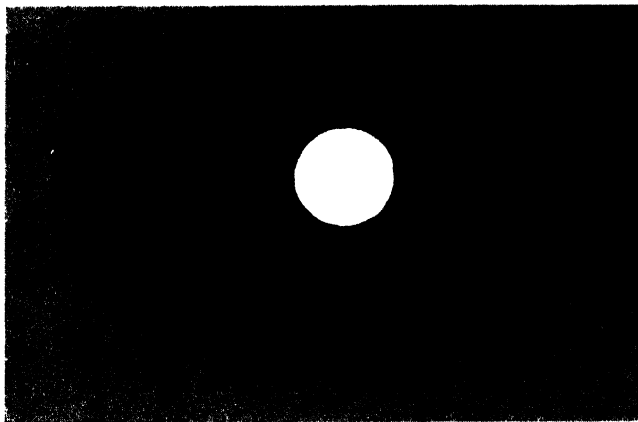



Fig. 73. Orientated embedding of silver amalgam in the ramie fibre. In addition to the fibre diagram of cellulose (broad interference spots) a fibre diagram of silver amalgam (narrow interference spots) appears (from FREY-WYSSLING, 1937a).

amalgam. If mercury is precipitated in the fibre from an alcoholic solution of sublimate, a dichroitic colouring is obtained which does not give rise to an X-ray diagram, because the mercury is present in the liquid state. Treating the fibres afterwards with a solution of silver nitrate, one obtains X-ray diagrams showing interferences of silver amalgam (Fig. 73) in addition to the diagram of cellulose. The silver amalgam crystallises in submicroscopic hexagonal needles which all run parallel to the fibre axis, for, instead of a DEBYE-SCHERRER diagram one obtains a *fibre diagram* of silver amalgam. This proves the presence of submicroscopic canals in the fibre.

Fig. 74. Ramie fibre stained with silver in the ultra-microscope (from FREY-WYSSLING, 1937b).

It is much more difficult, however, to get informations about the dimensions of these pre-formed capillaries. For, the size of the gold crystals embedded varies with the velocity with which they develop in the capillary system. Furthermore, the metal rodlets causing the dichroism are so large that they can easily be shown in the ultra-microscope (Fig. 74). Their rod-shape is betrayed by the different intensity of the light scattered in lateral irradiation according as the vibration of the linearly polarised light is parallel or perpendicular to the fibre axis (FREY-WYSSLING, 1937b). Consequently, the crystals or primary particles measured by means of X-rays must have clustered together to form rod-like aggregates or secondary particles (Fig. 75), widening the capillaries in doing so. Whether this occurs already when the crystals

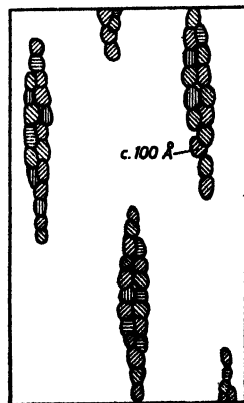



Fig. 75. Ultrastructure and shape of the submicroscopic silver rodlets in the fibre.

are growing cannot be decided. Presumably, however, they can develop fairly freely, since they do not acquire the rodlet shape of the capillary system until they are collected in the secondary crystalline particles. We must therefore take it as proved that apart from the intermicellar spaces in which the water penetrates when the fibre swells, still coarser pre-formed inhomogeneities exist.

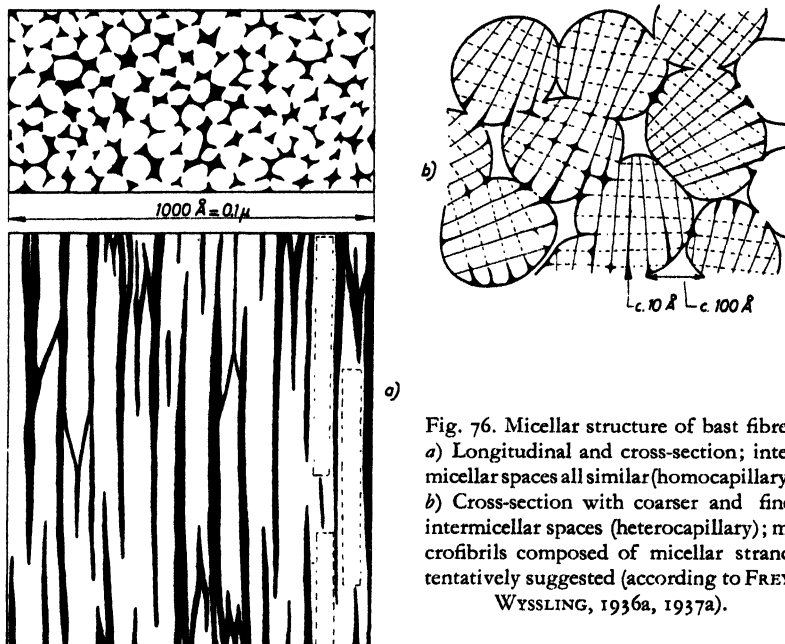



Fig. 76. Micellar structure of bast fibres. *a*) Longitudinal and cross-section; intermicellar spaces all similar (homocapillary). *b*) Cross-section with coarser and finer intermicellar spaces (heterocapillary); microfibrils composed of micellar strands tentatively suggested (according to FREY-WYSSLING, 1936a, 1937a).

There is, however, no fundamental difference between the finer and the coarser submicroscopic spaces, since the interstices can be widened or split into a continuous range of sizes. In swollen rayon the intermicellar spaces are probably uniform. Native fibres, however, seem to possess a less regular system of capillary spaces. There are long-shaped submicroscopic regions containing chiefly fine crevices, and which are therefore only accessible to swelling media such as water and salt solutions. These regions are designated as *microfibrils*; they possess a more or less *homocapillary* structure (Fig. 76a). In between these micro fibrils, however, interfibrillar capillaries must occur in the form of wider canals, in which larger molecules such as dyes and incrusting material are deposited. The intermicellar capillary system of ramie fibres is, therefore, *heterocapillary*; the smaller spaces (of the order of 10 Å) and the larger ones (of the order of 100 Å) communicate freely, and the ones become merged into the others. The microscopically visible fibrils must also contain both categories of spaces, because as a rule they can be dyed like the whole fibre, and they represent therefore aggregate bundles of the invisible submicroscopic micro fibrils.

Stretching experiments. A subject which has become of special importance in the studies of gel structure is the X-ray analysis of the *process of orientation* in stretching experiments. By way of example we shall briefly go into the phenomena observed in the stretch of regenerated cellulose fibres obtained from viscose.

It is possible to make isotropic cellulose fibres from viscose (HERMANS and DE LEEUW, 1937). The X-ray diagram of these fibres consists of DEBYE-SCHERRER rings. If the orientation of the micellar strings is completely random, photometric measurements show the intensity along each ring to be constant. If now the isotropic fibres are stretched, the micellar strands are oriented. With increasing stretch, the X-ray diagram changes into a sickle diagram and finally into a fibre diagram (Fig. 69)

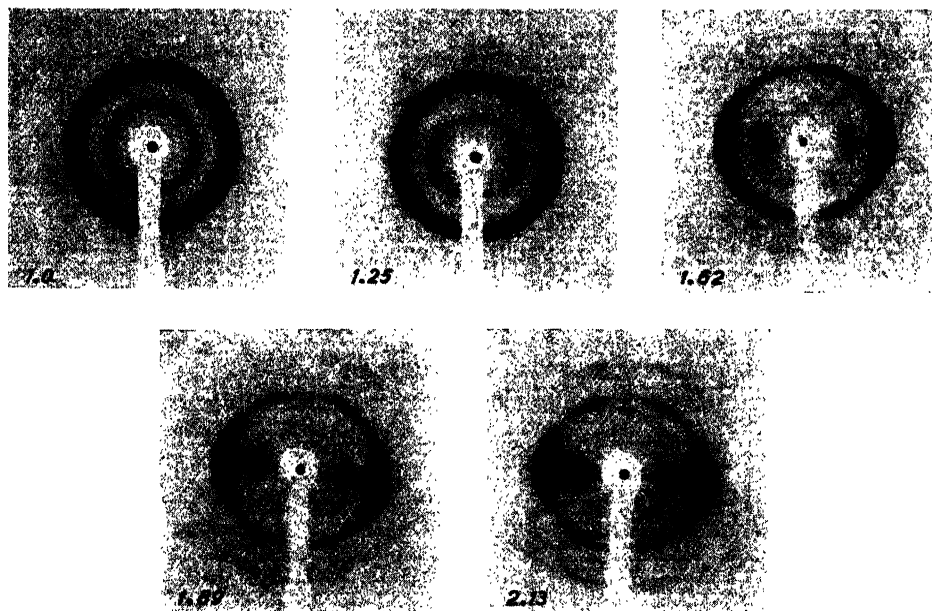



Fig. 77. X-ray diagram of HERMANS's threads, gradually stretched. The numbers give the degree of stretching (lenght of streched gel/original length). (According to KRATKY, 1940.)

when the orientation becomes complete. If, at a given degree of stretch, one measures the intensity along the interference sickles corresponding to the equator interferences in the fibre diagram (paratropic interferences), the *frequency* with which the different orientations of the micellar strands occur can be derived from the decay of the intensity from the equator towards the poles. In fact, the intensity depends on the number of lattice planes which take part in the reflexion of X-rays. It is possible in this way to determine experimentally the distribution function of the orientations of micellar axes.

If the distribution were one which covers a sector with uniform density (Fig. 66a), as was assumed on p. 63, the sickle interferences would be circular arcs with sharp boundaries, extending over an angle dependent on the angle of scattering in the sector. As shown by Fig. 77, however, the density in the sickle decreases very gradually towards the poles, and the distribution function is a very complicated one: the micellar strands which enclose a small angle with the direction of the stretch are more frequent than those which form a large angle with this direction, and this distribution is a function of the degree of stretch (HERMANS, KRATKY, and TREER, 1941). In order to explain the distribution curves found experimentally (intensity in dependence on angular distance from the equator), and their change with the degree

of stretch, KRATKY (1940) has made two different assumptions with regard to gel structure and has calculated how the distribution changes in the stretching process. Comparing these theoretical curves with those obtained experimentally, it is possible to decide which of the two hypotheses is the most likely.

The first limiting case considered by KRATKY (1933, 1940) conforms to the older ideas about gel structure, assuming rod-shaped "freely suspended micelles", which are independent of each other (Fig. 61b). Their orientation in the stretching process is achieved, as it were, by the flow of liquid (swelling medium) which turns the randomly distributed rodlets into positions which are parallel to the direction of the stretch. On this assumption the distribution of the micellar orientations can be calculated for any degree of stretch (i.e., final length divided by original length of the gel). Advanced parallel arrangement of the rodlets is only reached at high degrees of stretch. A number of highly swollen gels of cellulose esters (cellulose amyl oxalate, rinitrocellulose) show a behaviour which is in conformity with this theoretical distribution.

On the other hand, it seemed surprising at first, that in the case of relatively low degrees of swelling (between 1.5 and 2) neighbouring micelles do not disturb each other's movements and behave according to formulae which have been derived for particles freely suspended in a large amount of liquid. To explain this, KRATKY (1934) came to the conclusion that the arrangement of micellar rods is not completely random, but that there must exist *short-range order* (i.e., *order in small regions*). This means that if small, submicroscopic regions are considered, a certain parallel arrangement is found [1]. At some distance, however, the arrangement becomes gradually more and more disturbed, so that in a gel volume of microscopic dimensions already all possible orientations are found. This is shown by Fig. 78. Consequently, when indicating the dispersion of orientations in Fig. 53b or 56a, it must be borne in mind that neighbouring particles are almost parallel. An entirely different orientation is only found at a certain submicroscopic distance as a result of gradual changes in orientation. In the much coarser microscopic dimensions this means, however, that all anisotropy effects are neutralised as if a random criss-cross arrangement existed.

Fig. 78. Short-range order of short rod molecules (according to HERMANS, 1941b).

The principle of short-range order makes it intelligible that also gels of a low degree of swelling when stretched can behave as if their particles were freely floating micelles. In fact, the movement of each particle is very similar to that of a neighbouring one: there is no steric hindrance as would be the case if the arrangement were an irregular one. The introduction of the principle of short-range order does not suffice, however, to completely explain the behaviour of gels when stretched. For, the extensibility of these gels would have to be unlimited, and it should be possible to deform them to fibres of arbitrary length, even in those cases where the degree of swelling is low.

In the cellulose fibres mentioned, prepared by HERMANS, this is impossible. One must therefore take the point of view that the micelles are not freely movable but are

[1] The voluntary parallel arrangement of rod-shaped particles is not confined to colloid matter. It occurs also in pure liquids and real solutions, where physicists speak of short-range order (ZERNIKE 1939; STUART, 1941; PETERLIN and STUART, 1943). Taking an arbitrary molecule, its immediate neighbours are more or less ordered as regards distance and orientation.

interlinked by junctions (FREY-WYSSLING 1936a, 1936c) or hinges (Fig. 79). This assumption of complete interlinking of the structural elements in the gel is designated by KRATKY as second limiting case. Here again, there exists short-range order, and the picture arrived at (HERMANS, 1941b) corresponds more or less to the one given by us (compare Fig. 56). In other words, the orientation takes place as if chains consisting of rigid links and movable but inextensible hinges were stretched by pulling at the ends. On this assumption a distribution function for the orientation in network systems can be derived. A striking result of this theory is, that a completely parallel arrangement of all micellar strings would be reached at a degree of stretch 2 (100% stretch). This is not in keeping with the observations, since HERMANS' cellulose fibres, especially when highly swollen, can undergo a stretch of several times 100%. One must, therefore, assume that in reality neither the first nor the second limiting case is realized. The behaviour is intermediate between those corresponding to the two extreme cases calculated, i.e., the micelles are not freely suspended but they are interlinked to form micellar strands. The junctions present, however, are no fixed indissoluble hinges which completely prevent the micellar strands or parts of these strings from gliding past each other. Rather must the cohesion be due to forces which at certain points can be overcome by the orientating forces, so that a "flow in small regions" takes place.

Fig. 79. Short-range order in a gel of interlinked micelles (from HERMANS, 1941b).

c. *Swelling of Gels*

If isotropic gels are submersed in a swelling medium, they swell uniformly in all directions. If a certain orientation of the micellar strands prevails, however, the swelling is anisotropic, i.e., different in different directions. The anisotropy of swelling of starch grains has induced NÄGELI (1858) to consider the structural units (micelles) of the gel as submicroscopic rodlets.

Intermicellar swelling. According to NÄGELI, the swelling medium penetrates between the rodlets which we now designate as micellar strands. In many cases X-ray analysis has confirmed this view, since the X-ray diagram often does not change in the swelling process, so that apparently the crystalline regions remain unaltered (e.g., cell walls and cellulose gels).

The swelling medium penetrating between the string-like structural elements causes the system to inflate laterally. For this reason swelling is always largest in directions perpendicular to the direction of orientation of the micellar texture, and is almost zero along the fibre axis if the fibre texture is ideal. The arrangement of the micellar strands can therefore be derived from the anisotropy of swelling, or conversely, the anisotropy of swelling or de-swelling to be expected can be computed from the optical anisotropy measured (STEINBRINCK, 1906; ZIEGENSPECK, 1938).

If it is assumed that the microfibrils of native fibres, made up of polyhedral micellar strands, possess a more or less circular cross-section (Fig. 76b), these can be idealized

as circular cylinders. It is then found that in the completely dry state 9.3 volume per cent of intermicellar empty spaces must occur between the strings (HERMANS, 1938). This value tallies approximately with the average empty space (8.5 %) obtained from determinations of double refraction and density (FREY–WYSSLING and SPEICH, 1942), showing that in sharply dried fibres the microfibrils are fairly closely packed. Gels in which the colloid portion is crystallised imperfectly, so that a large amount of amorphous substance is present, swell much more strongly than well-crystallised fibres. For, the swelling medium is able to penetrate into the unordered regions, causing them to swell. Nevertheless it does not succeed to solvate the individual chain molecules in the ordered regions.

Intramicellar swelling. If, however, the affinity between the swelling medium and the chain molecules is stronger than the binding forces in the chain lattice, the swelling medium will penetrate into the lattice and widen it. This widening can be followed by means of X-rays; it often leads to the disappearance of the interferences. In that case the chain molecules are completely solvated and if they are not kept together by valence bridges (p. 47), unlimited swelling can take place which will gradually lead to the dissolved state of a sol.

In many cases, however, swelling media react with the side groups of the macromolecules, causing a change in the chemical character of the high polymer chains. This applies, for instance to the esterification of solid cellulose (nitration, acetylation, FREY-WYSSLING, 1936d). If the changed chain molecules cannot be solvated by the penetrating swelling medium, the result is a lattice of the newly formed substance, and no unlimited widening of the chain lattice takes place. This phenomenon too can be followed by means of X-rays, since the new chain lattice shows new interferences, while the original ones disappear. These conversions are designated as *permutoid* or *topochemical* reactions, because the reacting groups undergo chemical changes within the crystal lattice itself without dissolution of the molecules. The characteristic feature of these reactions lies, therefore, in the fact that chemical changes take place in the solid state, in contrast to the classical formula: *corpora non agunt nisi fluida.*

Intramicellar swelling clearly demonstrates the great similarity between swelling and dissolution. As has been shown by KATZ (1924), in both cases the same physico-chemical phenomena take place (heat of swelling, volume contraction and swelling pressure as a result of solvation), the only difference being that swelling occurs very slowly because of the slow Brownian movement of the macromolecules. And if in some way or other these form a network, only limited swelling takes place (swelling maximum) and the state of a sol is not reached.

De-swelling. Most gels encountered in nature possess a certain degree of swelling. On drying, the behaviour depends on the properties of their gel frame. If this possesses meshes with fixed contours, such as, for instance, silica gels, the decrease in volume does not correspond to the loss of water. The dry system is a porous body, i.e., it has changed into an air-containing *aerogel.*

If the gel framework is flexible, however, the meshes will gradually close on continued de-swelling till finally the micellar strings touch on all sides. The result is a horny, brittle *xerogel* without perceptible porosity. The drying process of these xerogels is very problematic. If we assume the gel to be isotropic, it must possess a randomly arranged gel frame. If we would apply this principle of randomness also to amicroscopic regions (Fig. 55a), the framework obtained when the molecular or micellar strands approach each other would be a loose structure with numerous

interstitial or intermicellar spaces. In that case the xerogel would possess a smaller specific weight than the crystalline substance and it would have a white and untransparent appearance as a result of the light diffraction caused by the air-containing spaces. This only applies, however, to aerogels, whereas xerogels solidify to completely transparent glassy substances. If the density of the crystalline micellar strands is determined by means of X-rays and compared with the specific weight of xerogels, the discrepancy found is of the order of magnitude of only 10% (HERMANS, 1938), whereas a dried mass of micellar strands would represent a more airy structure with a much smaller density. Examples of xerogels are gelatin and celloidin.

We are therefore compelled to pretend that in the drying process the contours of the gel frame are folded (compare Fig. 81b) or, since this hypothesis meets with great difficulties, to assume the existence of short-range order. Because of this short-range order of the micellar strands one can imagine continuous strings intersecting the whole gel (Fig. 56). The orientation never changes abruptly; deviations from parallel alignment are only gradual. Following such a continuous string or micellar strand in an isotropic gel, one finds a curve; neighbouring strings are approximately parallel (Fig. 87b, c). De-swelling causes the strings to approach each other. If the distance between them shall remain the same at all points, this must lead to a decrease in the radius of curvature (HERMANS, 1941b). It follows from this that on the assumption of short-range order the gel is capable of shrinking uniformly in all directions until the structural elements are close-packed, without the occurrence of kinks in the micellar strands (Fig. 78).

Discrepancy in the density of dry gels. The transparent brittle state of dry xerogels (dried glue, gelatin foli, horny celloidin, etc.) has led HERMANS and VERMAAS (1946) to compare these substances with glasses. In the manufacturing of glasses the rapid cooling of melts prevents the unwieldy molecules of quartz, silicates, borates, etc. from getting time to crystallise. The glassy amorphous state is, therefore, characterized by a similar molecular framework as the gels with amicroscopic framework, i.e., with chain molecules as structural units. Glasses possess a somewhat lower density than crystals of the same compound, since the closest packing of the molecules is attained in the crystal lattice only. For instance, the difference between the densities of butyl alcohol $CH_3CH_2CH_2CH_2OH$ in the crystalline and in the undercooled glassy state amounts to 6%. Gels with micellar structure contain ordered crystalline regions of micellar strands next to less ordered more or less amorphous regions. For the latter HERMANS (1946) assumes an amorphous glassy state. The gel consists therefore of crystalline and glassy amorphous parts. If the densities of the crystalline and the amorphous compound are known, the amount of crystalline material in the gel can be calculated. Using the reciprocal densities, i.e., the specific volumes, the following holds good: $x\,\varphi_{(cr)} + (1 - x)\,\varphi_{(am)} = \varphi$, where φ = experimentally determined specific volume of the gel, $\varphi_{(cr)}$ = specific volume of the crystalline part, $\varphi_{(am)}$ = average spec.vol. of the amorphous part, x = the fraction of crystalline material.

Substituting 1.55 for the density of the fibre (determined in toluene), 1.59 for that of crystalline cellulose and 6% less for amorphous cellulose (compare butyl alcohol), one finds for ramie fibres x = 0.54. With the aid of slightly different values HERMANS (1946) calculated x = 0.61 for ramie fibres and 0.18 — 0.32 for regenerated cellulose. In other words, only 1/5 to 1/3 of the cellulose in rayon fibres is crystalline. Whereas this result is quite acceptable, the amount of crystalline cellulose in ramie is

likely to be greater than 60%. Otherwise the difference between the birefringence of ramie fibres and that of crystalline cellulose ought to be greater than actually determined (FREY-WYSSLING and SPEICH, 1942; according to our measurements it amounts to 4.4% and, based on the double refraction 0.0705 of crystalline cellulose according to HERMANS, to 7.3%).

HERMANS objects to the opinion that dry xerogels are considered to be porous bodies, since in the case of glasses one also does not speak of submicroscopic spaces, in spite of the fact that the density is lower than that in the crystalline state. This comparison however does not seem quite justified to me, since certain liquids (such as water, alcohols and aldehydes in the case of cellulose) are capable of penetrating into xerogels, whereas this does not occur in glasses. Thus, clearly, there must exist a difference in the order of magnitude of the "empty spaces" present. In the swollen state xerogels decidedly possess a loose structure, and it is not likely that the micellar framework loses this structure completely upon drying. This is more likely to occur in molecular frameworks. Here the empty spaces shrink, and form interstitial spaces which do no longer possess the character of submicroscopic pores. It is therefore intelligible that in the poorly crystallised rayon fibres porosity disappears to a great extent in the drying process; all the same, even these fibres contain about 5% of empty space (Fig. 80). In native fibres whose incrustrations have been removed, a complete closing of the structure would hardly be possible. Otherwise it would be inconceivable that the density of ramie determined in toluene amounts to 1.55 while the result of accurate measurements of mass and volume gives only 1.39. Furthermore, an inner reserve of space is necessary to explain the great flexibility and torsion capacity; otherwise these fibres would be as brittle and elastic as glass fibres.

Fig. 80. Increase in volume of swelling isotropic (regenerated) cellulose threads (from HERMANS, 1946). Abscissa: absorption of water. Ordinate: specific volume (1/density). φ_0 specific volume of dried threads 0.66, φ_{cr} specific volume of crystallized cellulose 0.63. The water absorption increases linearly, but the volume does not; from this an apparent volume contraction of water results.

Double refraction of swollen gels. In the swelling process isotropic imbibition liquid penetrates between the anisotropic micellar strands. In this way the rodlet birefringence of gels is enhanced, for it follows from the formula given on p. 59 that if the other circumstances are kept constant, this birefringence acquires its maximum value when the relative volumes of rodlets and imbibition medium are equal ($\delta_1 = \delta_2$). The intrinsic double refraction, however, is inversely proportional to the volume so long as it is permissible to assume that no change in micellar orientation occurs as a result of swelling. If the intrinsic double refraction of the dry gel is called i-Do and the degree of swelling is q (volume of swollen gel/volume of dry gel), then,

according to KRATKY and PLATZEK (1938), the total double refraction of the swollen gel t-Do amounts to:

$$\text{t-Do} = \frac{\text{i-Do}}{q} + \text{r-Do}.$$

Consequently, if the intrinsic double refraction i-Do of the dry gel is known and the total double refraction t-Do of the swollen gel is measured, the rodlet birefringence r-Do of the swollen gel can be calculated. It is therefore possible to measure rodlet anisotropy in gels capable of swelling, provided it is taken into account that only those points must be combined to curves, which result from measurements

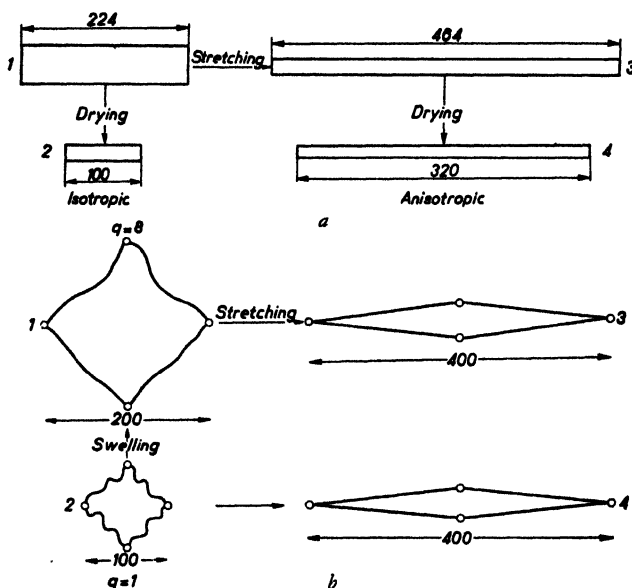



Fig. 81. *a*) Connection between swelling and streching. 1 swollen isotropic cellulose thread, 2 dried, 3 streched and 4 dried. The anisotropy and by this the texture of 4 is only dependent on the degree of streching as referred to state 2, but independent from the degree of swelling of state 1. *b*) If the gel strands are folded in state 2, stretching furnishes the same mesh length, whether it is performed directly (state 4) or by the detour of swelling (state 1 and 3). q degree of swelling; o junctions (according to HERMANS, 1941c).

in imbibition liquids giving rise to similar degrees of swelling. Otherwise one would obtain complicated kinky curves devoid of regularity instead of smooth WIENER curves (compare Fig. 64). If the gel consists of crystalline micellar strands mixed up with amorphous regions, as in cellulose gels, the optical behaviour of the swelling gel is composed of two effects: that of a molecular mixture superposed by a compo nent of rodlet double refraction (HERMANS, HERMANS, and VERMAAS, 1946).

Apart from rodlet double refraction, a further form of birefringence can occur when liquids penetrate between the amorphous chain molecules. This is attributed by VERMAAS (1941, 1942) to oriented adsorption of the penetrating molecules. It might also be due, however, to a change in the "intrinsic anisotropy" of the chain molecules

caused by the swelling medium, such as that occurring in sols when the refractive index of the dispersing medium is changed (SADRON, 1937; FREY-WYSSLING, 1943b).

Stretch of swollen gels. Dry gels can only be stretched to a limited extent, viz., to a degree of stretch of about 2. For this reason, to attain high degrees of stretch of the order of 10, the gel must first swell. In experiments of this kind one must therefore take into account two different parameters, to wit the degree of stretch v and the degree of swelling q. Whereas stretching brings about an orientation of the micellar strings, swelling results rather in desorientation. The phenomena are, therefore, very complicated.

HERMANS has found that the anisotropy of swelling (ratio between longitudinal and lateral swelling) of regenerated cellulose can be represented by a unique curve if, instead of the degree of stretch v, one uses the degree of stretch v_t which is obtained if both the unstretched and the stretched fibre are dried and the lengths of these two dry fibres are compared. The procedure is represented in Fig. 81a. Instead of a series of different curves for various degrees of swelling, we then obtain a unique curve which is independent of the degree of swelling. It has not yet been possible to fully elucidate the physical meaning of this characteristic degree of stretch v_t. HERMANS (1941c) has discussed a possible explanation, represented in Fig. 81b. Assuming that the contours of the meshes are folded in the drying process, the strings are stretched when the degree of stretch is a maximum, and the length of the meshes in the completely stretched state would be the same in both cases. HERMANS himself, however, criticizes this scheme, because it would apply at best to molecular frameworks with kinky chain molecules, and also because in those cases a folding-up such as that indicated in Fig. 81b without the formation of new junctions is very unlikely. In fact, we have only reproduced this scheme to show in what manner complicated problems in micellar theories may be discussed if one makes use of the assumption of junctions.

d. *Electron Microscopy*

Electron rays. The electrons which are sent out by a cathode are electrically charged negative particles with a mass of 1/1840 of that of a hydrogen atom. The range of these electrons in air is very short, because they are absorbed or scattered by atoms or molecules which they meet on their path. All investigations with electron rays must therefore be carried out in vacuo. On account of their electric charge they can be made to deviate from their straight trajectory by means of electric or magnetic fields. Bundles of electron rays can therefore be focused by electric coils in much the same way as light rays by lenses. This makes it possible to form images with electron rays according to the laws of geometrical optics (literature reviewed in ZWORYKIN and coll., 1945; BURTON and KOHL, 1946).

In as far as the electron rays represent a stream of particles, they can hardly be compared with light rays or X-rays. They have the remarkable property, however, to possess at the same time the character of waves. They can be deflected by crystal lattices and, like X-rays, give rise to interferences. An electron ray represents, therefore, a corpuscular ray and a wave train at the same time! The wave length λ of electron rays depends on the voltage applied to the cathode tube; λ is inversely proportional to the root of the tension. In the case of light waves the velocity of propagation in vacuo is independent of the wave length. This does not apply to electron rays, for, besides lowering the wave length, an increase in tension also results

in a greater velocity of the electrons. This velocity may become as high as 10^{10} cm/sec, i.e., 1/3 of the velocity of light. Since electron microscopy operates with very high tensions, the electrons are "rapid", i.e., rich in energy. At a tension of 57 kV the wave length amounts to about 5.10^{-10} cm = 0.05 Å (BORRIES and RUSKA, 1939a). This is one twentieth of the wave length of hard X-rays (about 1 Å) and one two hundredth of the wave length of soft X-rays (about 10 Å). In spite of this extremely small wave length and in contrast to X-rays, electrons have no penetrating power, since the electrons are already totally absorbed by layers of solid substances of a thickness of 0.1 μ. When passing through an object they lose part of their energy and leave it with a somewhat smaller velocity, i.e., with a changed wave length depending on the energy loss in the object. This means that the electron beam originally monochromatic becomes polychromatic, and images from electron lenses show not only spherical but also chromatic defects as light microscopic images do.

The electron microscope. Since the resolving power of the microscope depends on the order of magnitude of the wave length of the light used, one might expect a tremendous improvement in the resolving power of a X-ray microscope in comparison to the ordinary microscope. That dream could not be realized, because lenses for X-rays do not exist. The possibility of focusing electron rays has however brought the possibility to construct a short wave microscope. (MARTIN 1938; BORRIES and RUSKA 1939b; ARDENNE, 1940a, b; ZWORYKIN, 1940, 1941; ZWORYKIN, HILLIER, and VANCE, 1941; BORRIES, 1941; INDUNI, 1945).

The electron microscope operates according to the same principle as the ordinary microscope. The light source is replaced by a source of electrons. Usually this is a hot cathode, although it has the disadvantage of being rather sensitive to imperfections in the vacuum. For this reason INDUNI (1945) has constructed an electron microscope with a cold cathode which is less sensitive. The electron rays emitted are focused by a condenser coil and directed towards the object (Fig. 82). An object coil behind the object projects a real magnified image of the object, in the same way as an object lense in the ordinary microscope. In analogy to projection microscopy, this real image is magnified again and projected onto a screen by a projection coil, comparable to the ocular. Since electron rays are not visible, one uses a fluorescing screen, which lights up in proportion to the intensity of the incident irradiation, thus giving rise to a visible image. Since photographic plates are not only sensitive to ultraviolet and X-rays, but also to electron rays, the fluorescing screen may be replaced by a cassette for plates if microphotographs are to be taken.

Fig. 82. Comparison between light microscope (at right) and electron microscope (at left) (from INDUNI, 1945).

The whole path of the rays must lie in vacuo, which is maintained by means of a vacuum pump. For this reason the objects must be introduced from the atmosphere into the evacuated apparatus through a special sluice. The electron image on the fluorescing screen is observed through a window at the side. As in a projection drawing microscope, the source of the rays is in the upper part of the apparatus, and the object is irradiated from above, giving a projected image at about the height of the table. Fig. 82 gives a comparison with the ordinary microscope according to INDUNI's description (1945).

So long as the objects investigated have a thickness of more than 0.1 μ, the image in the electron microscope is formed in the manner of shadow images. Objects whose thickness is considerably less (order of magnitude 0.01 μ = 100 Å) transmit electron rays. In this case the theory of image formation meets with the difficulty that the electrons are slowed down and therefore change their wave length. The occurrence of an image is explained by observing that the electrons are deflected from their rectilinear trajectory by the atoms in the object, in much the same way as a small celestial body which gets into the sphere of attraction of a star. If now the object lense possesses a small aperture, the electrons which are deflected do not reach the image, and the object appears darker than the background. Since heavy atoms deflect electrons more strongly than light ones, metallic colloid particles appear darker than organic particles, which often furnish a very faint contrast[1]. In order to obtain images rich in contrasts, the aperture of the object lense must be as small as possible.

On the other hand, however, a small aperture is unfavourable for the resolving power of the microscope, for, according to ABBÉ's theory of image formation the resolving power increases with the aperture and reaches a maximum when this becomes ~ 1. Such electron optical lenses, however, cannot be made, for, like uncorrected light optical lenses they produce imperfect images, which are improved if only the central part of the lens is used. The present quality of electron lenses can be compared to that of the optical lenses at the time when ABBÉ began to eliminate their spherical and chromatic defects.

The necessary blindage of the scattered light and the defects of the lenses require very narrow bundles of electrons with apertures of only 0.001 to 0.003. As a result of the small apertures a applied, the resolving power d is not so overwhelming as could have been expected from the extraordinarily small wave length. As calculated for the ordinary microscope (probably ABBÉ's theory cannot be applied without alterations to the electron microscope, but interestingly the results are plausible), the resolving power amounts to $\lambda : a = 0.05$ Å/0.002 = 25 Å. This minimum, however, is only seldom reached. Usually the resolving power amounts to about 50 Å[2]. This is near to the smallest gold particles which have been demonstrated in the ultramicroscope (60 Å). Instead of luminous points, however, true images are obtained. The illusion of imaging molecules cannot be realized at present. Even in the case of macromolecules which are of the same order of magnitude as the resolving power, great difficulties are encountered in the realization of clear images (BOYER and HEIDENREICH, 1945), unless these organic particles are especially prepared (Fig. 86).

[1] It is often possible to enhance the contrasts by introducing heavy atoms such as iodine (HUSEMANN and RUSKA, 1940) or tungsten (tungsten phosphoric acid: HALL, JAKUS, and SCHMITT, 1945) by way of "electron dyes".

[2] See Report of the Electron Microscopic Soc. of America's Committee on Resolution, *J. Appl. Phys.*, 17 (1946) 989.

In spite of this, the success of the electron microscope is revolutionary. Considering the hard work needed to increase the resolving power of the ordinary microscope from dry systems with $d = 0.5\ \mu$ to quartz immersion systems for ultraviolet light with $d = 0.1\ \mu$, we cannot sufficiently express our admiration on realizing how the resolving power has been increased by a factor of about a hundred by the discovery of the electron microscope!

The small aperture of the objective coils is related to the great *focal depth* of electron optical images. This depth determines the ratio between the layer thickness in the object imaged sharply and the resolving power. In the ordinary microscope with large aperture the focal depth is only about 1, which means that a section of several μ thickness can be analysed into successive optical sections at different levels by means of the micrometer screw. In the electron microscope, however, this ratio is about 1000. This is a draw-back for the spatial analysis of the object. It is of great advantage, however, to the sharp focusing of the image and to obtaining stereophotographs (ARDENNE, 1940b; MÜLLER, 1942; HEIDENREICH and MATHESON, 1944).

The similarities and the dissimilarities between ordinary and electron microscopy have been compiled in Table XIV according to H. RUSKA (1941).

TABLE XIV

PROPERTIES OF ORDINARY AND ELECTRON OPTICAL IMAGES

	Light rays	Electron rays
Wave length	800–200 mμ	About 0.005 mμ
Penetrating power	Fairly great	Small
Kind of image	Shadow image (absorption) *Optical image* (refraction, diffraction)	*Shadow image* (absorption) Optical image (diffraction, interference)
Contrasts are caused by differences in	Refractive index	Density
Contrasts enhanced by 1. Change in the object 2. Decrease in wave length	 Dyeing Ultraviolet rays	 Impregnation with heavy atoms (J, Wo) Greater velocity
Focal depth	Small, ca 1	Large, ca 1000
Most favorable magnification	Up to 1000	Up to 20000
Resolving power	200 mμ	2.5 mμ

Whereas in the ordinary microscope the optical image caused by refraction and diffraction is the most important one, and shadow images are only observed as an exception, in the electron microscope the situation is reversed. As a result of the thickness of the object, the electron images are primarily shadow images; the optical phenomena of diffraction and interference in transparent thin objects have not yet been unambiguously accounted for. It seems that the resolving power is a function of the thickness of the object.

After removal of the ocular from an ordinary microscope, one can observe the phenomena of diffraction by microscopic fine structures. Similarly, the electron microscope may be changed into an apparatus which forms electron diffraction spectra if one removes the system of pole shoes of the projection coil (E. RUSKA, 1940). The electron diffraction diagrams obtained have the appearance of X-ray diagrams; they are only formed if a crystal lattice is present in the object.

Technique of raising preparations. (RUSKA, 1939, FREY-WYSSLING, 1946). Since the penetrating power of electron rays is small, it is difficult to find adequate specimen holders. The most suitable holders are nitrocellulose films of submicroscopic thickness. These can be made by spreading a drop of a collodion solution in amyl acetate on water, which is saturated with amyl acetate. On evaporation of the amyl acetate we obtain a nitrocellulose film which in favourable cases has a thickness of only 10 mμ.

When investigating suspended objects (bacteria, viruses, colloid particles), a drop of the suspension is left to dry on the specimen holder. Only dried objects can be brought into the apparatus, because the exposure must be made in vacuo. This excludes the observation of living organisms in the electron microscope, and it is also impossible to image cytological objects in their natural swollen state condition. If the dried particles do not give enough contrast to be imaged, as in the case of colloidal macromolecules, the objects must be dyed, as mentioned in Table XIV, or covered with a very fine film of metal. Fig. 86 shows molecules of hemocyanin clothed with evaporised gold cast down in oblique direction.

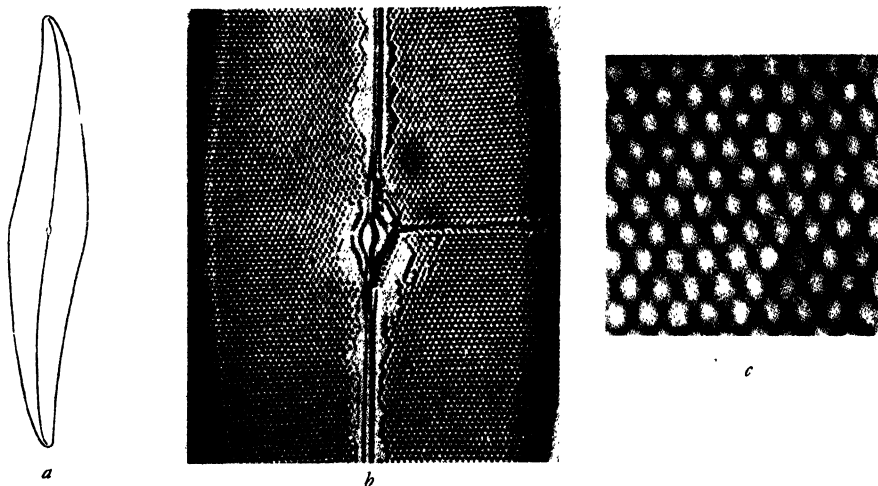
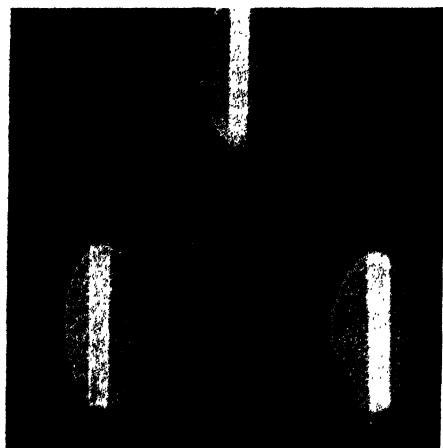



Fig. 83. *a) Pleurosigma angulatum* W. Sm. contour (from HUSTED, 1930); *b)* light microscopic image, image scale 1500:1 (from MICHEL, 1940); *c)* light microscopic image with numerical aperture 1.4, image scale 10000:1 (from ARDENNE, 1940b).

The methods described are appropriate for the investigation of corpuscular colloids. But in general those methods cannot be applied for reticular colloids with a coherent structure. For these objects a new microtechnique must be developped. It is true, that sometimes gel-solutions or gels can be dried on a specimen holder to be imaged (Fig. 87b). It is not yet possible, however, to prepare sections thin enough for the electron microscope, like those obtained in ordinary microscopy by means of microtomes. These sections would have to be of submicroscopic thickness, namely thinner than 0.1 μ. In some cases the thin edge of a wedge-shaped section is thin

a

b

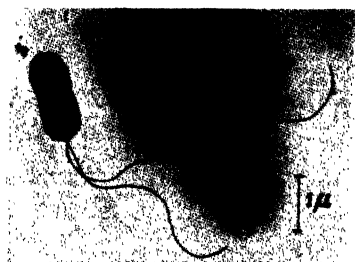
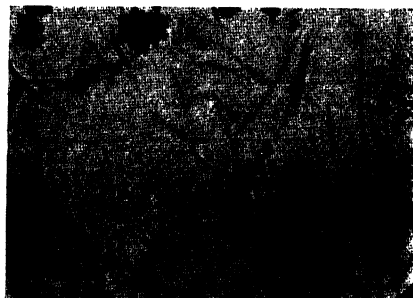
Fig. 84. *a*) Electron microscopic image of *Pleurosigma*, image scale 100000:1. *b*) Sketch of the spacial organisation of the silica wall; image scale ca 60000:1 (according to MÜLLER and PASEWALDT, 1942).

a

b

Fig. 85. *a*) *Bacterium pycocyaneum*, electr. microgr., image scale 8000:1 (from PIEKARSKI and RUSKA, 1939). *b*) Tobacco mosaic virus. Individual molecules and aggregates, electr. microgr., image scale 20000:1 (from KAUSCHE, PFANKUCH and RUSKA, 1939).

1 μ

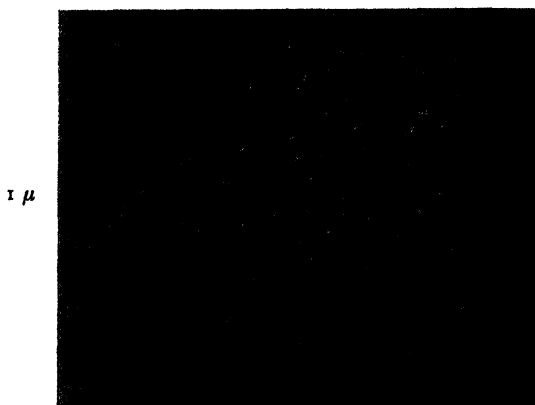



Fig. 86. Metallic shadow casting on hemocyanin macromolecules, electr. microgr. (from WILLIAMS and WYCKOFF, 1945).

enough for use in electron microscopy, but no images of large dimensions can be obtained in this manner. In thick objects such as cell walls and fibres the material can be rubbed small after letting it swell (WERGIN, 1942), or else they can be crushed into submicroscopic splinters in a vibrating ball mill. These splitters are suspended and finally dried on the specimen holder (HESS and coworkers, 1941). Some gels can be divided into submicroscopic flocculates by means of special vibrators (O'BRIEN, 1945). Ultrasonic waves have proved to be particularly suitable to this purpose. For example, this method allows of desintegrating microscopic fibres into submicroscopic fibrils (WUHRMANN, HEUBERGER, and MÜHLETHALER, 1946) without being under the necessity of destroying the structure in the vibrating mill.

The difficulties in making the preparations show that the electron microscope is more suited to the investigation of inorganic corpuscular colloids (submicroscopic powder, sublimates, smoke, dust, soot, etc.) than of biogels. If one succeeds nonthe-less in obtaining suitable preparations, new difficulties arise when these preparations are irradiated:

The electrons absorbed give a negative charge to the object, resulting in repulsive forces between the structural elements, which for instance may cause inflation of the fibrils at the points irradiated. The changes brought about by this effect, however, are as a rule less striking than those suffered by the object as a result of the *heat* developed. On absorption, the great energy content of the rapid electrons is for the greater part converted into heat. Silver and gold can be fused together or even molten completely in the electron microscope. It is intelligible, therefore, that organic compounds become charred if exposed too long. Many objects, such as bacteria for instance, appear brownish after exposure in the electron microscope, even if precautions are taken to spare them. The heat developed is, of course, the greater the thicker the object. Cooling of the object is not easily achieved, because in vacuo heat cannot be transferred by convection. All one can do is to remove the largest possible amount of heat developed by means of the metallic ring lying on the specimen holder. This is best done by laying a fine wire network over the ring of the specimen holder, and irradiating the object through the meshes of this network.

In spite of these precautions, biological objects often give rise to electron images which must be considered to be artefacts. Frequently they contain pores of sizes which are altogether out of proportion, surpassing many times the capillary spaces whose existence has been proved by indirect methods. Whereas in the beginning these holes were considered to be real (H. RUSKA, 1940), it was ascertained later that they were formed artificially by the action of the electrons. Examples of this kind show that great caution is required in the interpretation of electron optical images. Electron rays are not so innocuous as light rays, and for this reason an electron optical microphotograph is not always quite so true to nature as an ordinary optical image. The electron microscope admittedly gives direct information about the submicroscopic fine-structure, but the images obtained must first be checked on genuineness, and a submicroscopic structure cannot be considered as definitely established until all data compiled with indirect methods are in keeping with it.

Results of electron microscopy. After the minute description of the difficulties arising in electron microscopy of organic objects with a tendency to be charred we feel still more inclined to admire the great number of conclusive and far-reaching successes which in so short a time have been obtained in the biological field.

The improvement in the resolving power for structures invisible in the ordinary

microscope is most evident from the electron optical images which have been obtained from the silica wall of the diatomea *Pleurosigma angulatum* (Fig. 83a), the wellknown test-object fot the immersion objective of the ordinary microscope. While in the latter case the best objectives show three intersecting systems of lines (Fig. 83b), which at the utmost give a vague impression of a perforation (Fig. 83c; ARDENNE, 1940b), in the electron microscope Fig 84a is obtained. The surmised pores are clearly imaged with *sharp* edges and so far apart, that this "coarse" structure betrays what fine particularities can be resolved by the electron microscope. It is shown that the pores do not represent cylindrical canals running through the silica walls, but that the outer opening is in the form of a slit, while the inner one is elliptic and closed by a sieve membrane. Stereoscopic pictures show further that no canals but spacious caverns are present, whose outer openings represent the pores shown in the image (Fig. 84b, MÜLLER and PASEWALDT, 1942). Hence, the diatomea wall is not a massive structure, but it consists of an outer and an inner lamella, separated by submicroscopic spaces and connected by pillar-shaped buttresses (Fig. 84b).

Fig. 83c shows what was meant in Table XIV by "most favourable magnification". A microscopic image or a microphotograph can be magnified at will by projection, so that the magnification or better the *image scale* gives no unambiguous hold to compare different microscopes. Nevertheless there is a limit to the magnification of images in that the contours become vague when the image scale becomes too large. For this reason there exists a "profitable" magnification which is best maintained in microphotography and which is designated as "most favourable magnification". Strong magnifications of the microphotographic negatives obtained result in unsharp images as shown in Fig. 83c, where the systems of lines appear vague as a result of a magnification of 10000, which is five times the "profitable" one of 2000.

A greater sensation than that made by the elucidation of the submicroscopic structure of diatomea walls was created by studies in dried suspensions of bacteria and viruses. Fig. 85a shows a bacterium with submicroscopic flagellae (PIEKARSKI and RUSKA, 1939), while Fig. 85b shows the submicroscopic shape of tobacco mosaic virus. From photometer measurements in these photographs it follows that the virus filaments have a thickness of 15 mμ and a length of about 300 mμ (KAUSCHE, PFANKUCH, and RUSKA, 1939; compare Fig. 2 with data concerning the size, borrowed from STANLEY, 1938a, b). Since, according to measurements in the ultracentrifuge, the molecular weight of the virus protein amounts to 43 million, and X-ray data reveal a lateral identity period of 15.2 mμ (BERNAL and FANKUCHEN, 1937) in the hexagonal crystal of the virus protein (STANLEY, 1935, 1936), it follows that the rods represent *virus molecules!* Thus a direct image of organic macromolecules has been obtained. It has been proved that these molecules do not only show pathogeneous properties towards plants but also possess the remarkable property of auto-reproduction. This result, according to which single molecules may show properties which are attributed to living matter is of the utmost theoretical importance.

Suspensions of bacteria or viruses are corpuscularly dispersed sols which prove the applicability of the electron microscope in biology. Hopefully the biologist asks therefore what information the electron microscope may give on the structure of gels, among which we classify the protoplasm. By way of example we reproduce the electron optical image of a V_2O_5 gel serving as ultrafilter (ARDENNE, 1940b). One recognizes the reticular structure assumed on the basis of results obtained by indirect methods. The agreement with the scheme of Fig. 55a, proposed before the electron microscope had been discovered, is most striking. Results of this kind give us the

confidence required to apply the indirect methods to further research in submicroscopic structures, especially in those cases where the electron microscope as yet fails.

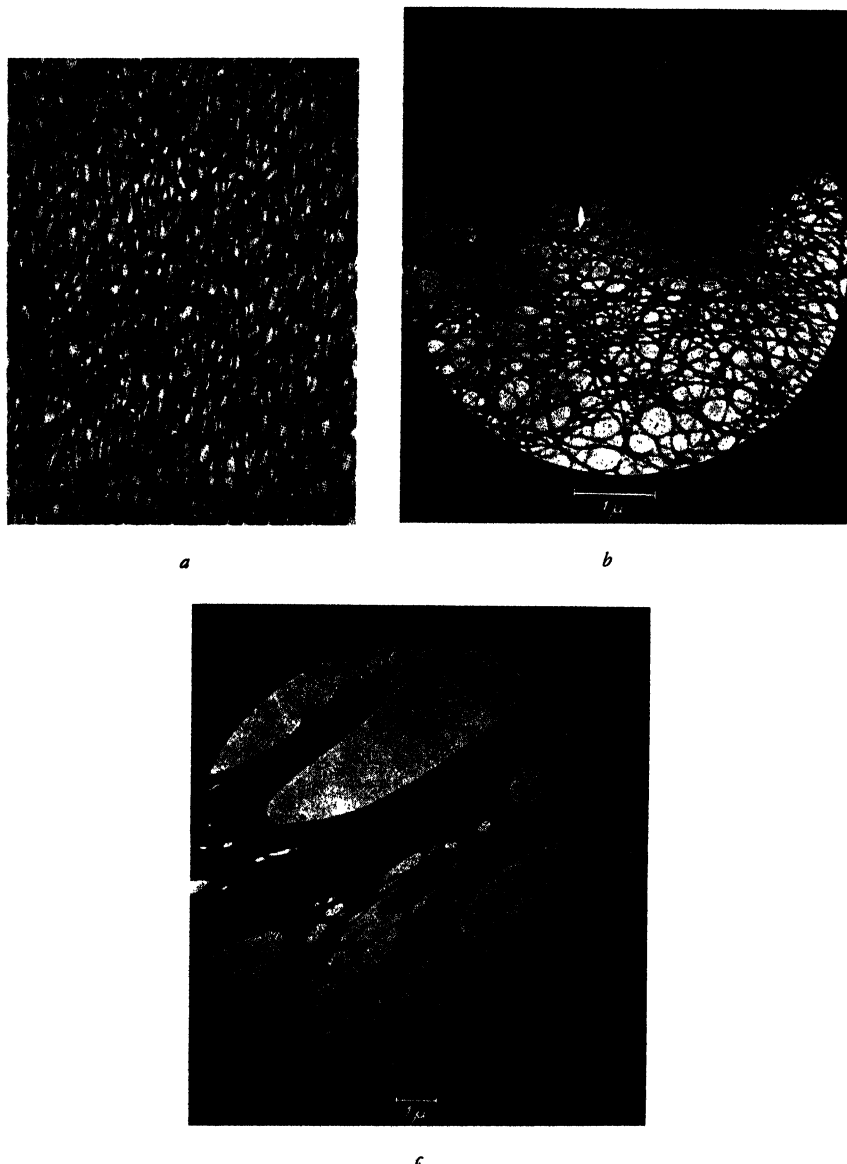



Fig. 87. Electron micrographs: *a*) Ultrafilter of vanadium pentoxide, image scale 35000:1 (from ARDENNE, 1940b). *b*) Dried gel of $V_2 O_5$, image scale 20000:1 (from FREY-WYSSLING and MÜHLETHALER, 1944). *c*) Cellulose film formed by *Bacterium xylinoides*, image scale 6000:1 (from FREY-WYSSLING and MÜHLETHALER, 1946).

Fig. 87a seems to contradict the principle of short-range order, since the gel strands show a criss-cross random arrangement. It should be taken into account, however, that a dry gel represents as it were a projection of the reticular texture, and

that further the great focal depth of the electron microscope causes gel strands separated in space to be imaged in a single plane. It is likely that the filaments crossing each other are not lying at the same depth in the gel, but that the greater part are oblique with respect to the image plane as is apparent from the lack of sharpness of numerous strand "ends". A stereoscopic view of Fig. 87b justifies the comparison of a gel with a wad of cotton wool. At various points ramifications of the gel strings are visible, showing that notwithstanding the apparent criss-cross arrangement of the gel strands there exists short-range order. The figure shows further that in the case of a gel thickness corresponding to an ultrafilter, all possible orientations occur in spite of the short-range order, so that there exists statistical isotropy, as indicated in Fig. 55. The curved micellar strands which are visible in Fig. 87b are particularly interesting because they favour branching of the strands.

As shown by Fig. 60 (blood fibrin, WOLPERS and RUSKA, 1939), the reticular structure postulated has also been found in biological gels. It can also be observed in concentrated virus sols, that increasing concentration causes the virus molecules to cluster together (gel-solution), and finally to form a felt-like gel frame, although as a result of the pronounced crystallising tendency of the virus protein a rather compact gel is formed instead of a loose framework with wide meshes.

Fig. 87c shows how gel strands can originate from a surface film which is homogeneous from an electron optical point of view. It is a flimsy cellulose film formed at the surface of a nutrient solution by *Bacterium xylinoides*. This film grows into a firm cellulose skin, but here it has been deposited in its first stage of growth onto a net serving as specimen holder, and has then been imaged in the electron microscope (FREY-WYSSLING and MÜHLETAHLER, 1946). One observes that cellulose filaments of about 0.02 μ (= 200 Å) thickness crystallise from the homogeneous film. Their thickness is of the same order as that of the microfibrils described on p. 73. It would be interesting to find the relations between these submicroscopic filaments and the micellar strands with a diameter of 50–60 Å, demonstrated by means of X-rays.

The images of other biogels obtained so far will be discussed in connection with the submicroscopic morphology of various constituents of the protoplasm. The recent literature on the subject has been compiled by MARTON (1945).

e. *Summary*

Gels with reticular structure are characterized by the existence of a framework whose constituent parts occupy definite mutual positions. The reticular state is fundamentally distinguished from the corpuscularly dispersed state by the fact that the strands or strings of the framework cannot be solvated completely and maintain certain *junctions*. If these junctions are released, the network character is lost. In this case the reticular gel which originally showed only limited swelling can change into the sol state via the gel-solution. As will be obvious from this definition, there exists a transitionary state between the reticular and the corpuscularly dispersed state. It is the task of the micellar theory to elucidate the morphological properties of the gel frame and the nature of the bonds in the junctions, which can be of quite different nature (see p. 97).

II. FINE-STRUCTURE OF THE PROTOPLASM

The great conquests in the field of structural chemistry have been realized by means of analysis and synthesis. Analysis provides information about the structural units, and with the aid of synthesis their position in the molecule is determined. Although no inner relationship seems to exist between chemistry and morphology, i.e., between our knowledge of matter and that of shape, this same procedure has been the method of research in morphology: detecting the structural units by analysis and determining their mutual position. The latter can be done by direct means both in the macroscopic and the microscopic domain and thus has no need of the indirect methods used in organic chemistry.

However, for the elucidation of the invisible submicroscopic structure of the protoplasm, in as far as it is not yet accessible to electron microscopy, analysis must again be combined with some kind of synthesis. It is true that this is not a matter of synthesis in the sense of organic chemistry. We can do no more than unite the structural units obtained by analysis in a scheme which enables us to explain the optical and physico-chemical properties of the protoplasm. Because of the extraordinarily complicated state of the inner morphologic structure of living matter, only a very incomplete solution of the problem is possible in this way.

In this situation one might be tempted to abandon the wearisome road of analysis and synthesis and simply to accept protoplasm as a given substance. This is, however, impossible for morphology as a branch of the exact sciences. For, so long as there are possibilities of research, morphology must from an inner necessity continue the analysis of living matter — even the sacredness of the human body has not been able to put a stop to it in former times. It is only when all possibilities of analytic dissection which the human mind places at its disposal have been exhausted, that it will bow in awe to the secrets of nature.

§ 1. CYTOPLASM

a. *Molecular Structure of the Cytoplasmic Compounds*

The description of the chemical composition of the cytoplasm (MENKE, 1938a) is given here only from the viewpoint of the molecular shape of its compounds (SPONSLER and BATH, 1942). The molecular structures concerned are known in principle, but an attempt at morphological synthesis of cytoplasm with the aid of these structural units can only result in a sketchy outline. Nevertheless, it is possible from this morphological point of view to explain the physico-chemical behaviour of cytoplasm to a certain extent.

Proteins (COHN and EDSALL, 1943). The basic substances of the proteins, isolated by means of hydrolysis, are α-amino acids which possess the structure given in Fig. 88a. Here R represents a group of atoms often containing a large number of C-atoms. To be exact, the NH_2- and COOH-groups should be bound to the C-atom as individual atom groups, as shown in Fig. 88b. It can easily be seen that two amino-acids can form a so-called dipeptide by eliminating water. If this process is repeated many times, a long polypeptide chain is formed, the ends of which have been left open in Fig. 88c. Like the paraffin chain it is kinked. The distance between two equivalent groups is 3.5 Å, as has been ascertained by means of X-ray analysis of crystalline fibre proteins. Only the >CO and >NH groups are similar along the whole length of the chain, while R differs according to the kind of protein and thus is responsible for the great variety in this class of substances. The zig-zag chain drawn in Fig. 88c can be considered as a relatively indifferent frame, which cannot be responsible for the chemical lability which we know the cytoplasm to possess. Its unusual reactivity is due to the side chains R.

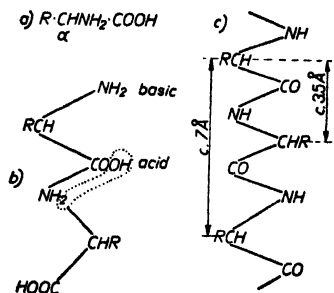



Fig. 88. Molecular structure of amino acids. *a*) Overall formula; *b*) principle of chain formation; *c*) polypeptide chain.

In chemical text books the amphoteric character of the proteins is often explained by the fact that amino-acids possess both an acid and a basic group (Fig. 88b). However, it follows from the structural picture of the polypeptide chain, that these groups disappear in the condensation process, thus losing their capacity for dissociation. If in spite of this the proteins clearly show acid or basic properties, this is brought about by the side chains which in their turn carry free COOH- or NH_2-groups. This happens when some members of the polypeptide chains consist of dicarbo-amino-acids or diamino-acids (Fig. 89).

The common amino-acids (valine, leucine, phenyl-alanine, etc.) cannot exercise a special influence on the reactivity of the proteins, but they provide these proteins with a pronounced lipidic character, since the ends of the side chains consist of methyl or phenyl groups (Fig. 89). In many cases, however, the terminal groups carry an alcoholic hydroxyl group (serine, tyrosine), on account of which a certain hydrophily is maintained.

A particularly important side chain is cysteine with its very reactive sulphydryl group. As will be shown later, this group very easily forms bridges between neighbouring polypeptide chains. In contrast to these compounds, capable of bonding and thus favouring further polymerisation, cyclic amino-acids such as proline can terminate the main valency chains and thus limit the apparently endless polypeptide chain molecules. The proline ring can, however, also be built in the peptide chain (see Fig. 147).

Considering the variety and the number of 20 amino-acids (besides some rare amino-acids, COHN and EDSALL, 1943) which have thus far been isolated from proteins and in view of the fact that these can occur as side chains at various points along the polypeptide chains, it becomes apparent what a many-coloured mosaic the protein components of the cytoplasm represent. It follows from Fig. 89, that the amino-acid configuration $-CH \cdot NH_2-COOH$ does *not* contribute to the character of the

mosaic, since it is only responsible for the peptide interlinking. The chemical behaviour of the polypeptides of the protoplasm is determined exclusively by the end groups of the amino-acids, to which often little attention is paid.

The possibilities of attachment of the side chains are so numerous that one

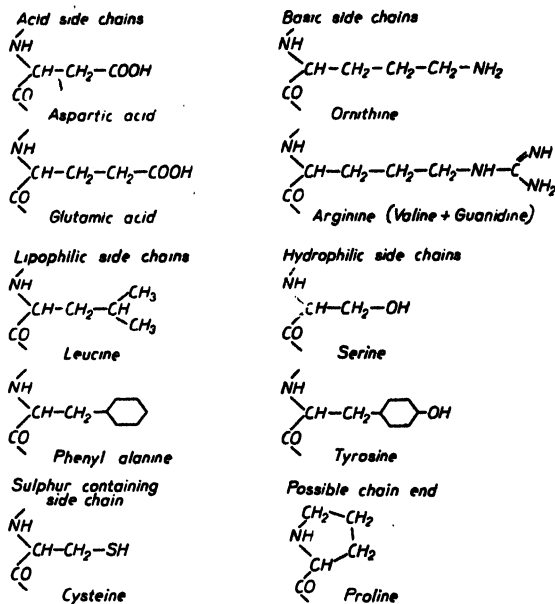



Fig. 89. Side chains R of the polypeptide chains.

would hardly have been able to decipher the structure of proteins if in certain cases they had not been particularly simple and subject to the principle of repetition. In silk fibroin, for example, they consist mainly of CH_3- or only H-groups (Fig. 144). Like the chain molecules of the polysaccharides such chains combine easily to form a crystal lattice. As will be obvious from Fig. 90, however, this is not possible if the side chains happen to be of quite different lengths and configurations. This explains why the complicated proteins of living matter never crystallise. By cooling or by plasmolysis, it is true, the proteins in the living cytoplasm of *Allium* epidermic cells can be made to arrange themselves into double refracting bundles (ULLRICH, 1936a), but these fall apart again at the return of normal conditions. Crys stallisation is hampered by condition- in space which are determined by the *side chains.*

These conditions can be compared with the ordering of bean- or pea-stalks. Whereas there is no difficulty in uniting a great number of smooth bean stalks into a bundle, it is not so easy to obtain a parallel order in pea stalks with their numerous twigs pointing sidewise; and if moreover the lengths of these twigs alternate in a quite irregular manner, the resulting structure becomes

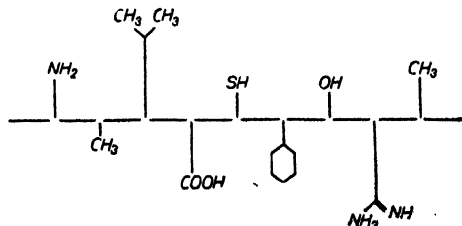



Fig. 90. Unequal lengths of polypeptide side chains R.

so spacious that it is almost impossible to bundle them together, as is the case with the polypeptide chains of the cytoplasm.

In addition to chains or fibrous protein molecules there exist *globular* molecules, in particular among the reserve proteins (see p. 216). It is possible that the one type of molecule can be transformed into the other one, although in a morphological sense they are quite different from each other; the mechanism of this process, however, is still unknown. This possibility of transition may be one of the causes of the lability and the physiologically polymorphous character of cytoplasm (see Fig. 155). In analyzing the frozen dried cytoplasm of potato tubers LEVITT (1946) finds a protein of the globular type with relatively low molecular weight (40–50000). It is difficult to say whether the cytoplasm of resting potatoes is converted to globular protein or whether this protein is to be considered as reserve material.

The polypeptide chains show a number of properties which single them out from the other substances of which the protoplasm is built up.

1. *The principle of repetition* which in biology we know as segmentation or metamerism. Most high polymer substances are built according to this principle. In the majority of these substances, however, identical monomer groups are repeated, whereas in the polypeptide chains the side groups R which occur in regular distances of 3.5 Å have different constitutions. Probably the typical side chains also repeat themselves regularly, but their period is much greater and is often not accessible to experimental analysis.

2. *The principle of specificity.* As a result of the great number of possible side chains R and their unlimited number of possible arrangements along the polypeptide chains, an infinite number of polypeptides is conceivable, which are distinguished only by slight alterations in construction. This difference in construction can result in a different chemical behaviour, which becomes apparent in the specific properties of the proteins.

3. *The principle of contractility.* The most striking property of polypeptide chains is their capacity to contract, as will be further discussed on page 211. The origin of the mobility of cells (plasm flow, cilia, contractible fibrils, etc.) must be sought in these molecular structural units and for this reason they form undoubtedly the most important structural elements in the fine-structure of plasm.

Lipids. The biological collective concept of lipids comprises all substances which are hydrophobic. This concept is therefore characterised by a negative property (insolubility in water) rather than by a positive one (solubility in organic liquids).

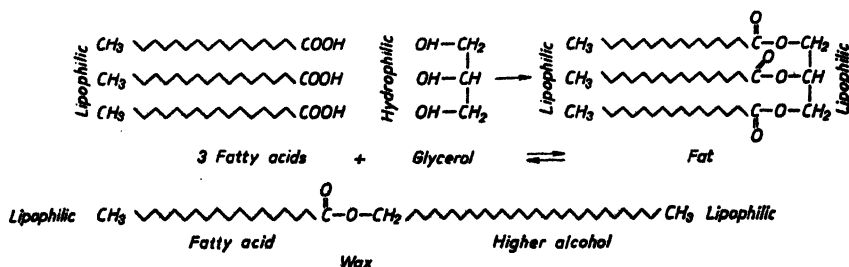



Fig. 91. Molecular structure of lipid chains.

For this reason it comprises different families of substances such as terpenes, waxes, fats, sterines, etc., of which the last two take part in building up the protoplasm.

True lipids are characterised by the fact that all their free end groups consist of typically lipophilic groups. This is especially obvious in the case of fats, which represent esters of the three-valent alcohol glycerol with fatty or oleic acids. As a result of the esterification, the hydrophilic groups of the original products are screened, as shown in Fig. 91. In the same way the hydrophile groups are masked in waxes which are formed by the esterification of higher alcohols with higher fatty acids. It is difficult to say why they are screened in the course of the metabolic process, but in any case these lipids contrast strongly with the hydrophilic compounds of living cytoplasm and if they are formed in excess, we observe the well-known phenomenon of the fatty degeneration of the protoplasm (lipophanerose). A certain proportion between hydrophilic and lipophilic compounds in living matter is essential.

In contrast to fats, most lipophilic compounds of the cytoplasm carry at least one hydrophilic group which serves to bring about the contact with the neighbouring hydrophilic groups. This applies in particular to the important group of the sterines (WINDAUS, 1923), of which Fig. 92 shows the complicated cholesterol $C_{27}H_{45}OH$. The molecule contains four rings and a double bond. The configuration given in Fig. 92 answers the chemical behaviour (ROSENHEIM and KING, 1932). According to X-ray analysis (BERNAL, 1932) the length of the molecule is 17–20 Å and its cross-section only 7.2 Å. The elongated form tallies well with the optical result, according to which solved cholesterol molecules can be easily oriented in a field of flow and like most rod-shaped molecules show positive birefringence of flow.

Fig. 92. Molecular structure of cholesterol; terminal group OH.

Phosphatides. Because of their solubility in ether, phosphatides are usually also counted among the lipids, but besides their lipid character they possess already a marked tendency toward hydrophily, which is shown by the sorption of water and the occurrence of myelin forms. Thus, phosphatides represent compounds which are intermediate between hydrophobic and hydrophilic substances and for this reason belong to the most important intermediates between the representatives of these two extreme groups in the cytoplasm. By way of example we may mention lecithin, which like fats consists partly of glycerol and fatty acids. In this case, however, only two OH-groups are occupied by fatty acids, while the third one is esterified by phosphoric acid and the latter in its turn by the amino-alcohol choline (Fig. 118).

Choline $HOCH_2–CH_2–N(CH_3)_3OH$ is a base whose hydroxyl group is attached to a methylated ammonium group. It would be conceivable that the three methyl groups give the end group $–N(CH_3)_3$ OH of the molecule a lipophilic character in spite of the hydrophilic OH-group. This, however, is not the case. For, interestingly, alkyl groups ($–CH_3$, $–C_2H_5$) bound to ammonium nitrogen show a hydrophilic behaviour (like methyl bound to oxonium oxygen, which makes pectic acid and methyl cellulose soluble in water, see p. 42). For this reason the ammonium end group tends to escape from the neighbourhood of the lipophilic end groups of the fatty acids. Consequently, the lecithin molecule resembles a tuning fork (Fig. 93), in contrast to fats which can be represented schematically by a threepronged fork

without a handle. The prongs of the fork represent the lipophilic pole, the handle of the fork the opposite hydrophilic pole of the lecithin molecule.

The phosphatides react with the protein chains of the cytoplasm by combining with either the lipophilic or the hydrophilic end groups of the side chains, as indicated in Fig. 93. This junction is not of a chemical nature, for the phosphatides can be extracted from the cytoplasm with ether. Non-the-less the phosphatide molecules occupy quite definite places, according to the character of the side groups in the polypeptide molecules. Lipids without hydrophilic groups, such as fats, can combine

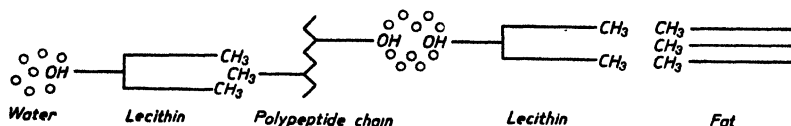



Fig. 93. Relation between polypeptide side chains and lecithin; o = water molecule.

only with the lipophilic side groups. For this reason their possible combinations with protein chains are limited. As shown in Fig. 93 they can only enter into relation with hydrophilic side chains by interposition of phosphatides or other intermediates.

The sterines possess a polar structure similar to that of the phophatides, but lecithin is superior in reactivity: of its two hydroxyl groups at the hydrophilic tail the one is acid (attached to phosphor) and the other basiç (attached to nitrogen). For this reason it can form salts with basic as well as with acid groups of the polypeptide chains. Phosphatides can therefore react with nearly all end groups occurring in the side chains of proteins. Here lies their great importance. To a certain extent all side chains of the polypeptide molecules are accessible to them. Sterines on the contrary are only capable of forming esters. Finally, for fats all side chains of the polypeptides, with the exception of the lipophilic end groups, are blocked. This shows clearly, how the possibilities of the lipids to enter into the cytoplasm become greater with their increasing hydrophilic character (FREY-WYSSLING, 1935c).

b. *Submicroscopic Structure of the Cytoplasm*

The molecules of the lipids and phosphatides are relatively small. They can be oriented by surface forces (compare myelin forms) or by the fixed side chains of the polypeptide chain molecules. On their own account they are not capable of building macromolecules of complicated structure. Among the components of the cytoplasm, only the protein chains possess this capacity. If the plasm possesses any structure at all, this must therefore be due to the polypeptide chains.

Now, the end groups of the side chains in the proteins react not only with the lipophilic or hydrophilic groups of other plasm compounds but also with the side groups of neighbouring polypeptide chains. Consequently, the separate chains grow together into a *molecular framework*. The places where the side chains are mutually connected will again be designated as "junctions" (see p. 47) and the nature of these points of attachment will now be discussed in more detail.

The theory of junctions[1]. The attractive forces between the side groups of neighbouring protein chains can be of quite different nature. Some of these possibilities are shown in Fig. 94. Lipophilic as well as hydrophilic groups can attract each other. Furthermore, salt-like or ester-like bonds can be formed between neighbouring

[1] In German: Haftpunkt-Theorie.

acid and basic or alcoholic groups, or even main valency bonds may be operative, forming ether-, acid amide- or sulphur bridges. Not all side chains take part in these reactions but a certain number with free end groups will combine with lipids, hydrophilic groups or water, as has already been described. Furthermore they form points of attraction for ions of the inorganic salts which, according to their charge, will gather round acid or basic groups. It is important that the end groups of many side chains remain free, for if they were all interlinked, the result would be a molecular framework of very small reactivity. It is known from the chemistry of saccharides that reactive groups which must be protected against conversion in the fermentative disintegration process are covered up by esterification with phosphoric acid (splitting of hexose diphosphoric acid; LEHNARTZ, 1942). It is, therefore, possible that the phosphatides play a similar rôle in the protein chains by occupying temporarily certain reactive side groups of the framework. Fig. 94 shows a molecular network with large meshes, in which lipids, water and inorganic ions find their appropriate places.

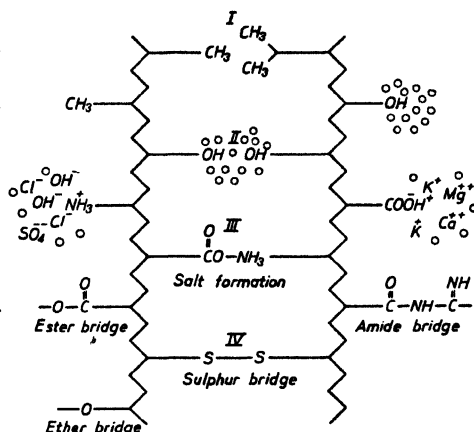
Fig. 94. Schematic representation of junction possibilities between neighbouring polypeptide chains in the cytoplasm; o = water molecule.

There exist four kinds of junctions keeping together the molecular framework formed by polypeptide chains. In Fig. 94 these have been numbered I–IV, and they can be characterised as follows:

I. homopolar cohesive bonds, i.e., mutual attraction of lipidic groups;
II. heteropolar cohesive bonds, i.e., attraction between groups with pronounced dipole character;
III. heteropolar valency bonds, i.e., formation of salts and esters;
IV. homopolar valency bonds or bridge formation.

We shall give a short discussion of the characteristics of these types of bonds, restricting ourselves intentionally to a sketch in diagram, to stress their importance for the properties of the cytoplasm.

I. *The homopolar cohesive bonds* are of the same kind as the forces which keep a paraffin crystal together. Very little is known about the causes of the attraction between lypophilic groups, for the electric charges in these substances are distributed so regularly that the resulting field of force is negligible, in contrast to dipole molecules. It has therefore been suggested that weak dipole moments are induced in the neighbouring molecules by periodic oscillations in the field of force, brought about by vibrations inside the electronic configurations (BARTHOLOMÉ, 1936). We know more about the energy of these bonds. As follows from Table IV, the cohesion between methyl and methylene groups is weakest among the cohesive forces. This kind of bond is therefore loosened by small amounts of energy and is therefore strongly *sensitive to temperature* changes. For this reason, paraffins, fats and waxes melt at relatively low temperatures in spite of their high molecular weight.

A similar behaviour is shown by the homopolar cohesive bonds between lipidic side groups of neighbouring polypeptide chains. By a rise in temperature this kind

of junction is easily loosened. Similarly, lipids and phosphatides which are attached to these groups become more mobile. This causes the living matter to liquefy to a certain extent: the rapidly decreasing viscosity of the cytoplasm as a function of the temperature is a well-known phenomenon (HEILBRUNN, 1930). Fig. 95 shows the rapid decrease of the viscosity of amoeba plasm between 10 and

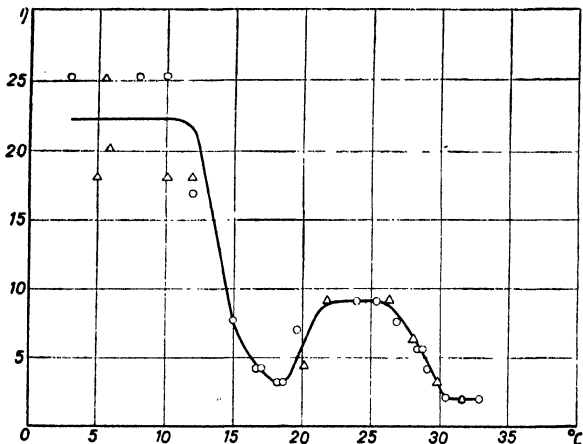



Fig. 95. Viscosity of the cytoplasm of the amoeba (according to HEILBRUNN, 1930). Abscissa: temperature in °C. Ordinate: viscosity (time in seconds, which a crystal enclosed needs to travel halfway through the cell under the influence of gravity).

20 degrees Celsius, which we attribute to the rupture of lipidic bonds in addition to the viscosity decrease of the intermicellar water. At temperatures beyond 20° C another process sets in, to wit a deswelling of the molecular framework at those spots where hydrophilic chain ends come together, resulting in some kind of solidification. At the same time, however, the rupture of lipidic junctions continues and at 25° C clearly surpasses the solidification brought about by dehydration. By raising the temperature still further, the curve should finally rise again since in that case the plasm would solidify as a result of deswelling.

Since in the physiological temperature range a rise in temperature would certainly not be able to rupture either heteropolar cohesive bonds or main valency bonds, it is permissible to attribute the change in viscosity of the cytoplasm primarily to the abolishment of homopolar cohesive bonds. The weakness of the homopolar cohesive bond is demonstrated by the extraordinarily small surface tension of plasm membranes (1 dyne/cm against nutrient, Table V), in comparison to water (71.6 dynes/cm against air, Table VI), where the surface is formed by heteropolar H_2O molecules.

II. *Heteropolar cohesive bonds* are of a quite different character. The underlying attractive forces are due to dipole moments (page 15), which are mostly so strong that they are designated as *secondary or rest valencies.*

Of recent years the semi-chemical character of the heteropolar cohesive bonds has come to the fore, since they are designated as hydrogen bonds or *hydrogen bridges* (PAULING, 1940). Wherever dipolar groups with hydrogen atoms situated in the periphery (OH-, NH_2-groups) are present, the possibility exists that these are attracted electrostatically by the local negative charges of the dipole groups of neighbouring molecules. To a certain extent the hydrogen atom acts as intermediary between the two molecules and connects them by forming some kind of bridge. This is repre-

sented in Fig. 96 for two polypeptide molecules running in opposite directions. The hydrogen atom is lifted somewhat out of its position in the original molecule and it looks as if part of the hydrogen valency is transferred to the neighbouring molecule. Clearly, this schematic representation of the "secondary valencies" gives only a very incomplete idea of the interactions of the two electric fields which attract the positively charged hydrogen atom with different field strengths.

If, for steric reasons, the heteropolar groups (OH, COOH,CHO,NH_2 etc.) of neighbouring molecules cannot sufficiently approach each other, their electric fields attract water molecules. Instead of hydrogen bridges, a hydration layer is shifted between them (Fig. 94) and it is obvious that with this kind of junctions the cohesion depends on the number of water molecules between the two end groups, i.e., on their hydration. For this reason heteropolar cohesive bonds are *sensitive to swelling.*

Fig. 96. Hydrogen bonds between polypeptide chains.

Swelling depends strongly on the presence of inorganic ions, in which case the so-called *ion series of* HOFMEISTER holds good (see HÖBER, 1922). Their influence on swelling phenomena can be explained morphologically on the basis of the diameter and hydration layers of the ions. GOLDSCHMIDT has calculated the diameters of the ions from the distances between the atoms in the crystal lattice, and the size of the hydration layers can be derived from the ion mobilities. For the monovalent kations, for instance, the following radii have been found (Table XV).

TABLE XV

ION RADII

	Li	Na	K	NH_4	Rb	Cs
In the crystal lattice according to GOLDSCHMIDT	0.78	0.98	1.33	1.45	1.46	1.66 Å
Derived from the conductivity at ∞ dilution	3.66	2.81	1.88	1.89	1.81	1.80 Å
Number of H_2O per ion, according to PALLMANN (1937)	10.0	4.3	0.9	0.8	0.5	0.2

Obviously the small ions have thicker hydration layers than the bigger ones. This is due to the fact that the water dipoles are attracted more strongly according as the distance between the centre of gravity and the surface of the ion is smaller. Fig. 97a shows a graphical representation of the water layers. It demonstrates how the ionic radii grow with increasing atomic weight while the water layers decrease.

If a gel swollen in water is imbibed with salt solutions, the penetrating ions will weaken the electric field of the hydrophilic dipole groups of the gel frame; consequently their hydration decreases, which results in *deswelling.* In the case of biogels this effect of shrinkage in neutral salts is observed only in rather concentrated salt solutions (from about ½ n upwards) which in most cases must be considered to be non-physiological. Deswelling by means of salt can therefore be used for preserving purposes (brining of meat) or for the salting-out of dissolved proteins.

The degree of deswelling depends on the radius of the hydrated ions as long as the other conditions remain constant. For instance, if dried agar powder swells in 1 n alkali chloride solutions (BRAUNER, 1932), the degree of swelling is smaller than in water, and it is found that in comparison with the other alkali ions, Li and

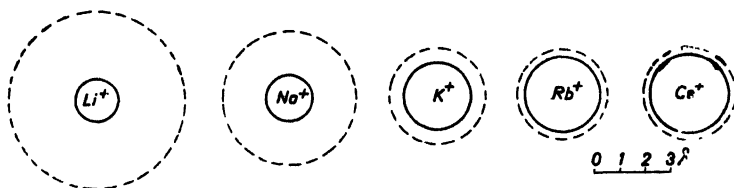
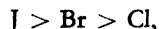



Fig. 97a. Hydration. Ions of the alkali series; hydration layer dotted.

Na ions result in a higher degree of swelling, in accordance with the series of Fig. 97a:

$$Li > Na > K > Rb > Cs$$

Using only potassium salts and varying the anion in the halogen series, one finds:

$$J > Br > Cl,$$

i.e., the more strongly hydrated Cl causes less swelling than the lesser hydrated J. This inversion of the influence of ion hydration shows that the influence of the ions on swelling phenomena is determined primarily by their charge. Biogenic gels, such as agar in the present case, usually possess a weakly negatively charged gel frame. For this reason the discharging effect of kations of equal valency is inversily proportional to their hydration. The effect of the anions is due to the fact that the discharging kations are accompanied by their gegenions. These lay the greater claim to the charge of the kations, the smaller their hydration. For this reason, the discharge of the gel framework by a given kation accompanied by J ions is less than if it were accompanied by Cl ions. In other words, for a given kation the water sorption of the gel will be the greater, the less hydrated the anion of the salt.

In many cases, however, gels do not swell less but more strongly in salt solutions than in water. This occurs if the gel framework possesses *ionogenic groups* as is the case with proteins. For example, the gel frame of gelatin, when imbibed with a neutral salt solution, shows a considerable negative charge as a result of the dissociation of COOH-groups. For this reason kations can be retained by adsorption; their hydration is larger than the dehydration of the gel framework, caused by the adsorption of the kations. Thus it becomes possible that the degree of swelling reached in salt solutions is higher than that in water.

Fig. 97b. Hydration. Influence of ions on the hydration of polypeptide chains; △ = difference in swelling.

Fig. 97b indicates how ions of equal valency can cause different degrees of swelling. An anionic side chain and a hydrophilic OH-group of a neighbouring polypeptide chain are related to each other. Both possess a hydration layer. If a Na ion surrounded by its hydration layer approaches this system it is held electrostatically, and a hydration equilibrium between the various

groups is established. If the Na ion is replaced by a much less hydrated ion like Rb, the latter is able to approach the anionic group more closely because of its smaller hydration layer. This results in a stronger discharge than in the case of the Na ion; the hydration decreases and the neighbouring polypeptide chains approach each other.

An explanation along these general lines becomes more difficult if bivalent ions such as Ca take part in these processes. Since bivalent ions carry two elementary charges, they can discharge negative proteins more strongly than monovalent ions. For this reason they usually cause deswelling of the protoplasm (decrease in permeability, increase in density and viscosity; CHOLODNY and SANKEWITSCH, 1933). In the case of the trivalent ions Fe and Al these effects are still more pronounced (tannage). One speaks, therefore, of a *valency rule* of deswelling, which says that the deswelling effect of ions increases with rising valency.

With increasing charge of the ions, however, also the hydration layer increases. The Ca ion, for instance, is more strongly hydrated than the K ion of the same size. Accordingly, $CaCl_2$ causes gelatin to swell to a greater extent, which can even result in the formation of a sol. In the same way the strongly hydrated Zn ion in concentrated $ZnCl_2$ solutions causes an unexpectedly strong swelling of cellulose. The valency rule is therefore not of general applicability to bivalent ions.

The valency rule asserts itself more clearly in HOFMEISTER's series of the anions

$$SCN > J > NO_3, Br > Cl > \text{acetate} \mid > SO_4 > \text{tartrate} \mid > \text{citrate}.$$

The trivalent citrate ion is a weaker swelling agent than the bivalent tartrate and sulphate ions and these last two are weaker agents than the monovalent ions.

In the case of positively charged proteins with kationic polypeptide chains, HOFMEISTER's ion series with respect to swelling phenomena are reversed, because the adsorption now refers to the anions. This inversion is particularly striking, if one succeeds in reversing the charge of a negative gel. For instance, for gelatin in a neutral or basic medium, in which the gel framework acts as an anion, the order in which ions further swelling is as follows

$$Li > Na > K > Rb > Cs$$

In an acid medium, however, in which the gel framework behaves as a kation:

$$Li < Na < K < Rb < Cs.$$

Now one would expect that in the isoelectric, i.e., uncharged state, the gel frame would show the same degree of swelling in all neutral chlorides, since in that case no electrostatic attractive forces are operating. This is, however, not the case; one finds so-called *transitory series* which are of special importance for biology:

$$Li > Na > K < Rb < Cs.$$

This result is not easily comprehensible after what has been said before. For if one plots the degree of swelling against the atomic weight of the kations, one obtains a descending curve in alkaline solutions (gel framework negative) and an ascending curve in acid solutions (gel frame positively charged; Fig. 98a). For this reason one would expect a horizontal line if the p_H of the swelling medium has been adjusted to the isoelectric state (I. E. S.) of the protein. However, the experiment yields a minimum curve in which K holds a special place.

By using ion models, however, it is possible to understand these relations too. It follows from the ion mobilities that the two ions in KCl are of equal size. For this reason they are adsorbed in the same way by an isoelectric framework. In LiCl and NaCl, however, Li and Na are adsorbed to a lesser extent than Cl because of

their large hydration layer. Consequently, the molecular framework assumes a weakly negative charge and is more strongly hydrated than in KCl. Conversely, in RbCl and CsCl the kations are more easily adsorbed than the Cl ions, which again results in a weak electric charge of the gel, accompanied by increased hydration (Fig. 98b).

Since the isoelectric state of the cytoplasm usually lies in the weakly acid region, kations have a discharging effect on it. Kations therefore as a rule cause less swelling

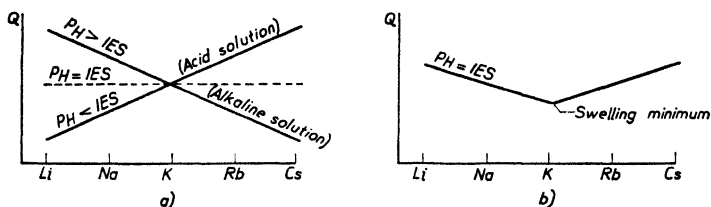



Fig. 98. Change in swelling of a gel frame consisting of polypeptide chains under the influence of chlorides of the alkali series at various p_H values; Q — degree of swelling. *a*) HOFMEISTER series; dotted line = behaviour expected at p_H = I.E.S.; instead one finds *b*) transitionary series.

than water. Anions on the contrary increase hydration as a result of their similar electric charge, so that often a considerable swelling of the cytoplasm occurs (cap-plasmolysis, p. 124) in particular with SCN, J, Br, but also with Cl which is held responsible for the swelling of the plasm of halophytes (STOCKER, 1928). In the series J, Br, Cl, F, chlorine often takes a similar optimal place as potassium in the alkaline metals and Ca in the alkaline earths (PIRSCHLE, 1930).

III. *Heteropolar valency bonds.* If all acid and basic groups in the cytoplasm exactly cancel, the isoelectric state (improperly designed as "isoelectric point" I. E. P.) is attained and nearly all properties of the plasm reach extreme values: the degree of swelling becomes a minimum, the danger of setting a maximum; the stability is low, the electric charge and the migration in an electric field become zero by definition, etc.

If then positive and negative end groups of the side chains occupy suitable positions with respect to each other, they can enter into saltlike bonds (Fig. 94 III). Their electric charges are neutralised and the hydration of the region in question

TABLE XVI

ISOELECTRIC STATE (I. E. P.) OF CERTAIN PROTOPLASTS (ACCORDING TO PFEIFFER, 1929)

		p_H
Bacteria:	*Bacterium coli*	12–13
	grampositive bacteria . . .	about 5
	gramnegative bacteria . .	2–3
Fungi:	*Fusarium*	5.4
Algae:	*Nitella*	4.4–9.6
Angiosperms:	*Hyacinthus* (root tip) . . .	4.3
	Lupinus, Pisum ,, . . .	4.3
	Rheum	4.5–4.8
	Solanum	6.4

is reduced to a minimum. The salt bonds cannot be changed or even loosened so simply by neutral salts as the heteropolar cohesive bonds. To this end more drastic

means have to be applied: the concentration of the H-ions, i.e. p_H, must be changed. The intermolecular salt bridges are then partly hydrolysed and a certain number of the bound carboxyl and amino-groups become free. If hydrolysis is achieved by H-ions, i.e., if the p_H of the liquid in which the cytoplasm is examined drops below the I. E. P., the dissociation of the free COOH groups is diminished, that of the amino-groups ($-NH_3OH$) is furthered. Thus the cytoplasm acquires a positive electric charge and behaves like a complex kation. Conversely, if the p_H of the medium is greater than the I. E. P. of the cytoplasm, the dissociation of the COOH groups is furthered and the cytoplasm becomes negatively charged, i.e., it acts like a weak anion. This occurs as a rule in neutral nutrients, since the I. E. P. of protoplasts is usually lower than 7 (Table XVI).

The isoelectric state determines the acidity at which the heteropolar junctions of the salt bonds are most effective. Any deviation of the p_H from this state results in a loosening of this type of bond.

Up to a certain extent also esterifications, i.e., bridges formed between alcoholic OH and acid groups of neighbouring polypeptide chains (Fig. 94 III), can be reckoned among the heteropolar valency bonds. Their firmness is dependent also on the p_H of the medium, since hydrogen ions are capable of hydrolysing and hydroxyl ions to saponify these ester bonds catalytically.

IV. *Homopolar valency bonds* are formed either by elimination of water (ether, glucoside and peptide bridges, Fig. 94 IV) or by splitting off hydrogen, i.e., dehydrogenation (methylene and sulphur bridges, Fig. 99a). The former still possess a certain polarity and can be hydrolysed under suitable conditions. Without the aid of enzymes this is no longer possible at physiological, but only at higher temperatures; compare, for instance, the hydrolysis of glucosides and proteins by boiling acids. This is of particular importance for the stability of the substances forming the carbohydrate framework and for that of the molecular chains in the cytoplasm which consists chiefly of peptide bonds. The purely homopolar valency bridges ($-CH_2-CH_2-$, $-S-S-$) can no longer be hydrolysed at all. Here the loosening of the junctions is achieved according to an entirely different principle, namely by addition of elementary hydrogen (*hydrogenation*).

cystine bridge: $\rangle CH-R-SH \quad HS-R-CH\langle \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \rangle CH-R-S-S-R-CH\langle$

methylene bridge: $\rangle CH-R-CH_3 \quad CH_3-R-CH\langle \underset{+2H}{\overset{-2H}{\rightleftharpoons}} \rangle CH-R-CH_2-CH_2-R-CH\langle$

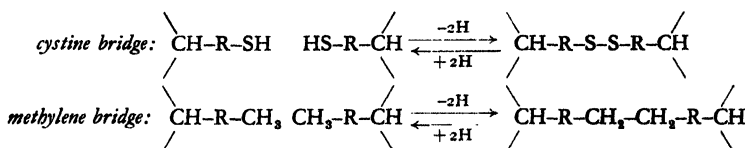



Fig. 99a. Bridges dependent on r_H.

At physiological temperatures water does not only in very small amounts dissociate into ions according to the scheme $H_2O \rightleftharpoons H^+ + OH^-$, but also, though admittedly to a still lesser extent, into the elements hydrogen and oxygen: $2H_2O \rightleftharpoons 2H_2 + O_2$. These gases develop a very low gas pressure, which for hydrogen we shall designate as tH_2.

If the partial pressure of hydrogen in the cytoplasm increases, the $-S-S-$ bridges tend to be hydrogenated, which causes rupture of the bonds (Fig. 99a). The cystine bridges can therefore absorb H_2 and for this reason act in the same way with respect to the partial pressure of H_2 as a buffer with respect to the concentration of H^+ions. These conditions have been investigated in particular in the case of *glutathione* (G).

This is a protein compound which can be split into glutamine, cysteine and glycine. It represents a tripeptide chain with the three amino-acids mentioned as side chains. However, whether it occurs in the cytoplasm as a free molecule or only as part of a much longer polypeptide chain cannot be decided at present. In both cases glutathione reacts according to the following scheme: $2\,GSH \rightleftharpoons GS{-}SG + H_2$. Thus, when sulphydryl groups occur in the side chains of the molecular framework of the cytoplasm (Fig. 94 IV), these can give rise to formation or dissolution of *cross-links*.

Methylene bridges cannot be formed with the same ease or, in any case, not in the laboratory, where methyl groups show a very passive behaviour. All the same it is known that in the dissimilation process one molecule of succinic acid can be formed out of two molecules of acetic acid by dehydrogenation (MOTHES, 1933). This succinic acid is then dehydrogenated further to fumaric acid, converted into malic acid, dehydrogenated to oxalo-acetic acid and finally, after decarboxylating this keto-acid, converted into pyruvinic acid. It thus becomes apparent that dehydrogenation plays an important part in the chemistry of fermentation. It is, therefore, likely that to a certain extent this also applies to the formation of methylene bridges between neighbouring polypeptide chains. It is known that in asphyxia the cytoplasm often liquefies; this may be due partly to hydrogenation processes, resulting from increased partial pressure of the hydrogen.

The hydrogen pressure in the plasm is characterised by its negative logarithm in much the same way as the hydrogen ion concentration. The p_H is derived from the product of the ionic concentrations $(cH^+)\cdot(cOH^-) = 10^{-14}$. Similarly, the product of the H_2 and O_2 partial pressures in water is constant. It amounts to $(tH_2)^2 \cdot tO_2 = 10^{-82}$, in which the pressures are expressed in atmospheres. Thus the H_2 and O_2 pressures are mutually dependent in the same way as the H^+ and OH^- concentrations. The hydrogen and oxygeh pressure or the so-called redox potential of a solution in water can therefore be characterised by a single number. For this purpose we choose the negative logarithm of the hydrogen pressure, which is designated as r_H.

If hydrogen is made to flow through a system under atmospheric pressure, the hydrogen pressure amounts to 1 at., or, written in exponential form: 10^0 at., which means that $r_H = 0$. On the other hand, if oxygen flows through the system, $tO_2 = 1$, and accordingly $(tH_2)^2 = 10^{-82}$ or $r_H = 41$. Obviously the r_H of a system can vary between 0 and 41. Small values of r_H indicate lack of oxygen, larger ones on the contrary are indicative of favourable aerobic conditions. r_H (like p_H) can be measured directly with the aid of a potentiometer (Fig. 99b) or indirectly with the help of suitable dyes (MICHAELIS, 1933) which lose colour at a certain r_H as a result of hydrogenation (for instance methylene blue and indigo). In Table XVII the analogies between p_H and r_H have been collected. The characteristic values of the r_H scale are apparent from the following list:

	r_H
1 at. O_2 (oxygen electrode)	41
air (1/5 at. oxygen)	40.7
hydrogen and oxygen pressure in equilibrium	27.3
H_2 pressure = $2 \cdot O_2$ pressure (middle of redox scale)	20.5
border of { aerobic life / anaerobic life	8
1 at. H_2 (hydrogen electrode)	0

Table XVIII gives a few r_H measurements in living cytoplasm (NEEDHAM, 1925, RIES, 1938). The values are not strictly comparable, since according to the equation $H_2 - 2\,el^- \rightleftharpoons 2\,H^+$ the value of r_H is a function of p_H. This dependence is apparent from Fig. 99b (according to BLADERGROEN, 1945). If the electric redox potential E (with respect to the platinum hydrogen electrode E = 0) and the value of p_H in the system are used as rectangular coordinates, the curves of constant hydrogen pressure (r_H) are sloping lines. If two of the three quantities: electric redox potential E, the exponent of hydrogen pressure r_H and the exponent of hydrogen ion concentration p_H are known, the magnitude of the third one can be read from the diagram in Fig. 99b. Since the redox system is only significatively determined by its electric potential E, it follows that in biological systems besides the

r_H value also the corresponding p_H value should be given. On this condition the r_H value may be identified with the redox potential, as is usually done in biology.

TABLE XVII

p_H AND r_H SYMBOLISM

	Actual acidity p_H	Redox system r_H
Starting point	hydrogen ion conc. cH^+	hydrogen pressure tH_2
Dissociation	$H_2O \rightleftharpoons H^+ + OH^-$	$2H_2O \rightleftharpoons 2H_2 + O_2$
Law of mass action	$cH^+ \cdot cOH^- = 10^{-14}$	$(tH_2)^2 \cdot tO_2 = 10^{-82}$
Exponent	$p_H = -\log cH^+$	$r_H = -\log tH_2$
Interval	p_H varies from 0–14	r_H varies from 0–41

TABLE XVIII

REDOX POTENTIAL (r_H) OF CERTAIN PROTOPLASTS (ACCORDING TO NEEDHAM, 1925)

	p_H	r_H
Sea-urchin egg	6.5	19–21
Amoeba proteus	7.6	17–19
Salivary gland of *Chironomus*	7.2	19–20

Just as the heteropolar valency bonds are strongest at a certain pH, namely at the I. E. P., there is an optimum value of r_H at which the homopolar valency bonds are the least endangered. It has already been pointed out how cystine bridges are broken down at high hydrogen pressures, i.e., at low r_H-values. At high values of r_H they are reestablished. A high r_H is, however, also capable of loosening bonds (oxydation). As shown by STAUDINGER (1937a, p. 13), the glucoside oxygen bridges of cellulose from a certain degree of polymerisation onwards are very sensitive to oxydation, so that the chains are easily degraded, for instance according to the scheme: $(C_6H_{10}O_5)_{2n} + O_2 = 2(C_6H_{10}O_5)_nO$

Similar sensitive ether bridges may be assumed to exist in the cytoplasm, so that not only too small a r_H but also too high a r_H may interfere with the bonds in the molecular framework.

Fig. 99*b*. Relation between redox potential E, hydrogen ion exponent p_H and hydrogen pressure exponent r_H. Abscissa: p_H-value of the system; ordinate: electric potential E of the system with respect to Pt-H_2-electrode (according to KLADERGROEN, 1945).

Apart from dehydrogenation, i.e., elimination of hydrogen, also the transfer of hydrogen atoms from one chain to a neighbouring chain may be responsible for bridge formation. ASTBURY (1936) and ASTBURY and WRINCH (1937) discuss two possibilities of bridge formation inside folded polypeptide chains of fibre proteins, and similar reactions may also be considered in the protoplasm. The hydrogen can be exchanged between neighbouring

keto and imido groups following the lactam-lactim tautomerism according to the abbreviated equation $> CO + HN < \rightleftharpoons > C(OH)\text{–}N <$, thus building a main valency bridge. In the same way bridges may be formed between keto and methylene groups by keto-enol inversion: $> CO + RHC < \rightleftharpoons > C(OH)\text{–}RC <$. Such inversions often occur quite easily, and in many cases it is impossible to decide which of the two forms is present. As regards the cytoplasm, this would mean that because of the possibilities discussed it would remain doubtful whether a bridge existed or not, i.e., its existence might be obvious at one moment and fail at the next, which would be in accordance with the great instability of the bond and with the mobility of the chains.

Summary. The *polypeptide chains* are to be considered as the structural elements of the cytoplasm. Their main chains are interlinked to form a fine molecular framework, whose junctions or knots can be disrupted by various quite different agents. A rise in temperature attacks in the first place the homopolar cohesive bonds or lipidic bonds. Dependent on their state of hydration, adsorbed salts affect the heteropolar cohesive bonds or secondary valency bonds; the p_H influences the heteropolar or salt bonds, and the redox potential is even capable of intervening, either as a constructive or as a destructive factor, with the homopolar valency bonds or bridgelike bonds. It is therefore very difficult to explore the structure of the cytoplasm experimentally, for it is scarcely possible to vary only a single one amongst these four factors, keeping the three other ones rigorously constant. A change in the temperature or the salt concentration will often cause changes in p_H and r_H, which in their turn depend upon each other. For this reason one can never be sure in an experiment whether some measure has not affected other types of bonds besides the group of points of attachment which one wished to investigate. In spite of the fact that it is practically impossible to keep the four types of junctions as neatly apart as in theory, the projected scheme might give a welcome survey of the various kinds of bonds which by their harmonious collaboration are responsible for the wonderful molecular framework of the protoplasm.

In case of *fixation* the aim is to preserve the molecular framework as true to nature as possible. This can never be done in an ideal way, since the usual means of fixation apply to quite different categories of the junctions. Alcohol has a dehydrating and hardening effect on the heteropolar cohesive bonds. In order to counteract the accompanying shrinkage, a swelling medium such as acetic acid has to be added. Its H-ions lessen the contracting action of the alcohol by hydrolysis of heteropolar valency bonds and by maintaining a certain state of hydration of the heteropolar cohesive bonds. Oxydising means of fixation like chromic acid and osmic acid affect bridges which are sensitive to r_H and thus solidify the labile homopolar main valency bonds. The tanning action of formaldehyde may be due to its capacity to form bridges between neighbouring polypeptide chains according to the same scheme as that which governs the polymerisation of oxymethylene. It is impossible to find a fixation mixture which does in no way affect the structure of the labile molecular framework. In spite of this, fixations which have been carried out correctly may not be compared with precipitations since no separation of phases takes place but only a coarsening of an existing structure. It is shown by the dyeing experiments carried out by DRAWERT (1937b) with varying p_H of the imbibing liquid, that the molecular framework after fixation still contains acid and basic groups capable of dissociation, although these groups are no longer screened off but freely accessible to dyes.

With the aid of the diagram of Fig. 94 some indications about the importance of the various elements for the structure of the protoplasm can be given. In the periodic system (Table XIX) all elements which are of importance to the life of plants lie on a line connecting carbon with the inert gas Argon. I have designated this line as *nutrition line* (1935d); only hydrogen and molybdenum (ARNON and STOUT, 1939) make an exception.

TABLE XIX

ELEMENTS WHICH ARE INDISPENSABLE FOR PLANT NUTRITION

Series	0	I	II	III	IV	V	VI	VII	VIII	0
1st period		H								He
2nd period	He	Li	Be	B	C	N	O	F		Ne
3rd period	Ne	Na	Mg	Al	Si	P	S	Cl		Ar
4th period	Ar	K Cu	Ca Zn	Sc Ga	Ti Ge	V As	Cr Se	Mn Br	Fe Co Ni	Kr
5th period	Kr	Rb Ag	Sr Cd	Y In	Zr Sn	Nb Sb	Mo Te	Ma J	Ru Rh Pd	X
6th period	X	Cs Au	Ba Hg	La Tl	Ce usw. Pb	Ta Bi	W Po	Re -	Os Ir Pt	Rn
7th period	Rn	-	Ra	Ac	Th	Pa	U			

In Table XIX the indispensable elements have been framed by drawn squares, whereas those which are found in nearly all plants but whose indispensability remains to be proved have been framed by dotted lines. C and N lie in the centre. These elements occupy a central position in the molecular framework too, since they form the polypeptide main chains. They may therefore be designated as chain building elements. The chains are built according to the scheme –C–C–N–C–C–N–. Notwithstanding its close relation to nitrogen, phosphorus does not occur as chain building element in this manner, but only in combination with oxygen (compare Fig. 113; –C–O–P–O–C–); as in the inorganic domain, it is always present in oxydised form as phosphoric acid. In the degradation of carbo-hydrates it acts further as protector of atom groups which should not be affected (hexose diphosphoric acid, phosphorus glyceric acid, etc.). It is possible that in the cytoplasm the phosphatides, which can combine with various groups of the polypeptide chains, render a similar service. The elements O and S of the VIth row are primarily *bridge building elements*, since they interconnect the C–N-polypeptide chains. Apart from this, oxygen can act as chain building element in the high polymeric carbohydrates, and conversely N and C are capable of bridge formation.

The elements of the first and second row: Na, K, Cu, Mg, Ca, Zn, and also Cl occur in the cytoplasm as ions and act as *hydration regulators*. They form no stable bonds but only heteropolar salt bonds with the molecular framework (metallic organic compounds like chlorophyl, haemoglobin, etc. are quantitatively of minor importance). The most propitious to the cytoplasm of plants are the ions K, Ca and Cl of the so-called Argon type (in animals Na takes the place of K). Both in mixtures and in pure solutions these ions are tolerated in concentrations at which other ions are detrimental to the cytoplasm structure. This would also explain why the nutrition line takes its course towards Argon. The higher valent elements B, Mn and Fe presumably enter into some relation with the plasm frame. As regards manganese and iron it is usually believed that their capacity to change valency is put to use in metabolism.

The most important part is played by the element hydrogen, both as ion and as element. It regulates p_H and r_H, thus preventing the molecular framework from solidifying, and maintaining its labile changeable state which is so characteristic for the protoplasm.

Physical properties of the cytoplasm. Sol properties. Many cytologists suppose the cytoplasm to be a liquid (RHUMBLER, 1898). HEILBRUNN (1930), for example, writes about the amoeba: "it is a tiny sac of fluid in motion" and CHAMBERS (1925) considers not only the cytoplasm but also the nucleus to be a liquid phase.

The flow of the protoplasm, the considerable fluidity, the relatively low viscosity, the great water content, the soft consistency, the convex shape in plasmolysis and other indications point to the sol character of the cytoplasm, i.e., to a state in which all submicroscopic particles can move freely with respect to each other. Most striking and always astounding is the *plasm flow*, and when seen for the first time this phenomenon will always convince the observer of the liquid state of the cytoplasm.

The merit of having characterized the aggregate state of the cytoplasm with the aid of physical laws is due to RHUMBLER (1914). According to his observation, the cytoplasm of the amoeba possesses 1. no measurable elasticity, 2. no perceptible compressibility at ordinary pressures and 3. it follows the capillary laws which are determined by the surface tension (minimum surface, constant contact angle, spreading on the surface of liquids, capillary rise). HARTMANN (1933, p. 37) has discussed these properties from the point of view of the then current colloid science. At the present time our picture will be somewhat different.

According to NEWTON's law, ideal liquids are completely free of inner elasticity: any particle in the bulk of the liquid can be moved at will without showing the slightest tendency to swing back into its original position. In the cytoplasm this condition is not fulfilled, for, as shown on p. 46, it possesses *structural elasticity* or *elasticity of flow*. The incompressibility should not be tested at "ordinary" pressures, but at high pressures where the low compressibility in comparison with solid bodies becomes apparent. If a living amoeba in its nutrient would be exposed to a *uniform pressure* of the order of magnitude required to prove incompressibility, the cytoplasm might not outlive the experiment. It would be destroyed, whereas it is the main property of ideal liquids not to undergo any changes in this experiment.

RHUMBLER's best arguments refer to the capillary properties of the naked cytoplasm, although by no means all cytoplasts assume a spherical shape or can be spread at will on the surface of another liquid; there can even be no question of a capillary rise except in selected special cases. However, even in those cases where the cytoplasm forms liquid drops, the capillary laws do not allow of drawing the conclusion that arbitrarily selected particles can be moved arbitrarily with respect to each other. This will be shown in the following discussion.

Consider a wad of thread-like algae. The threads can be moved at will with respects to each other, although they impede each other's freedom of movement as a result of their extremely anisodiametric shape. When transferring this microscopic model to the molecular domain, the threads become long chain molecules in a dispersing medium and a drop of this macromolecular sol would show all the capillary phenomena described. If the individualized algae threads of our model were replaced by the graceful reticular alga *Hydrodictyon* (OLTMANNS, 1922, p. 277), scarcely any change in the inner mobility of such a wad of algae would be observed. On a molecular scale this means that a drop which contains a coherent three-dimensional

molecular network instead of free chain molecules will not only assume a spherical shape but also show a constant contact angle and spread on the surface of suitable liquids. In spite of this, the structural elements of the network cannot move freely! The network is so flexible, however, that its shape within the drop is determined by the forces of surface tension. All the same we cannot speak of a true liquid, for, when static equilibrium is established, the drop is *inhomogeneous* not only at the surface but also in bulk. For this reason the experiments on the capillary rise of liquid cytoplasm are not conclusive.

The above shows that the occurrence of capillary phenomena gives no conclusive evidence for the existence of a true liquid. On the other hand, however, it has not been proved that the liquid cytoplasm possesses an organised structure. It has only been shown that the possibility of such a structure cannot be excluded.

The same holds good for the deviations from POISEULLE's law shown by liquid cytoplasm (see p. 46). Since this law does not apply to protoplasts (Fig. 52), the liquid cytoplasm must be a gel-solution. This does not yet imply a definite structure, although once more this possibility is not excluded.

Not so with the deviations from STOKES's law. According to this law, microscopically visible particles or bubbles in a liquid either fall or rise with *constant velocity* according as the density of the particle is larger or smaller than that of the liquid. This property can be used to measure the viscosity of liquids, since for a given difference of density and diameter of particle the velocity is inversely proportional to the viscosity. SCARTH (1927) has ascertained, however, that in cytoplasm the particles do *not* fall with constant velocity. It looks as if they meet with invisible obstacles; they fall therefore in a hesitant and jerky manner. According to SCARTH it has the appearance of hail-shot which is run through a brush heap. Again and again the falling particles meet with invisible strands, lose speed and change their direction. Accordingly the cytoplasm cannot be homogeneous but must be full of invisible fibres of a higher density. It does not possess a uniform viscosity, and the results derived from the fall method (HEILBRONN, 1914) represents some kind of average value. In PEKAREK's viscosity measurements (1930, 1932), which are based on the amplitude of oscillation of particles in Brownian movement, the inhomogeneity of the cytoplasm finds expression to a much lesser extent, because the oscillatory motion does not cover a large distance through the cytoplasm but can be studied at a fixed point.

The low values found for the relative viscosity of the cytoplasm cannot prove its true liquid state, even though they are considerably lower than the values for viscous true liquids (Table XX). For, a true liquid should in the first place be homogeneous in the physical sense and this certainly does not apply to the cytoplasm.

To sum up it can be said that the cytoplasm in its liquid state obeys neither the laws of NEWTON (PFEIFFER, 1937) nor those of POISEUILLE or STOKES (FREY-WYSSLING, 1940a). Although to cytologists it may have the appearance of a liquid, it certainly is *no true liquid* in the physical sense. We had better not attach too much value to its similarity to true liquids, for we should then have no possibility of penetrating into its submicroscopic fine-structure, since a liquid possesses a structure only in its surface. On the contrary, it is my aim to stress especially the *deviations* from the physical laws of liquids, since these deviations can give us a hold to elucidate the structural properties of the cytoplasm.

TABLE XX

RELATIVE VISCOSITY η

Water	1	
Cell sap:		
Stem parenchym of the *Vicia Faba* germ	1.9	(HEILBRONN, 1914)
Protonema of *Leptobryum piriforme*	1.9	(PEKAREK, 1933)
Epidermic cells of the *Allium Cepa* bulb	2	(PEKAREK, 1930)
Terminal vacuole of *Closterium* (see Fig. 103a)	2.5	(FREY, 1926c)
Cytoplasm:		
Amoeba	6	(PEKAREK, 1930)
Stem parenchym of the *Vicia Faba* germ	24	(HEILBRONN, 1914)
Red cell of man	30	(PONDER, 1934, p. 87)
Viscous liquids:		
Glycerol	87	(LANDOLT-BÖRNSTEIN, 1923)
Paraffin oil	92	
Castor-oil	1250	

Gel properties. Often the cytoplasm does not flow in liquid drops, but shows *plastic* properties. This in itself would not yet be sufficient to indicate a solid state; but besides it is *elastic* and to a certain extent possesses a *constant shape.* The result of plasmolysis is not always a liquid drop which is separated from the cell wall with a well-pronounced convex shape. On rapid dehydration with strongly hypertonic solutions the shape in plasmolysis becomes concave or angular, indicating a certain stability of shape of the cytoplasm in this state (PRUDHOMME VAN REINE, 1935). The inner elasticity can be demonstrated by suspending iron filings in the liquid

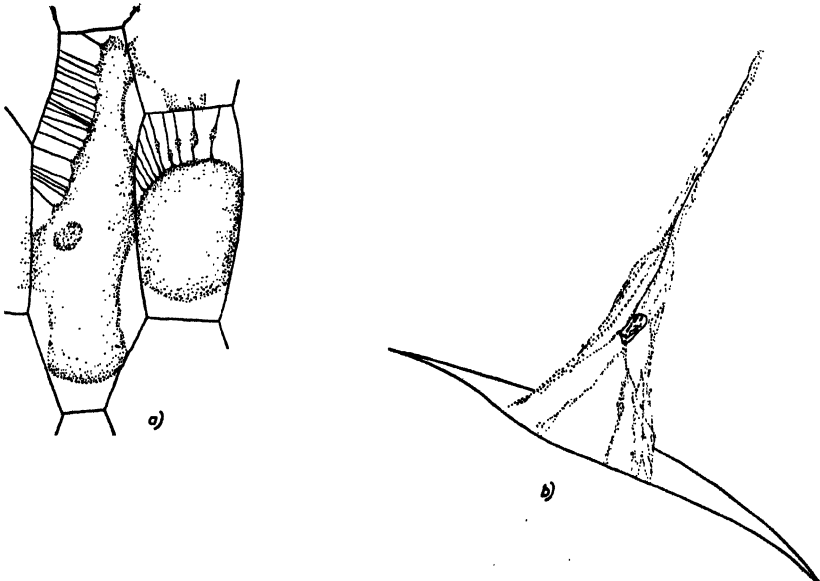



Fig. 100. *a)* Plasm strands of epidermic cells from the bulb of Allium, plasmolysed by $CaCl_2$ (according to KÜSTER 1935*a*); *b)* plasm strand of *Spirogyra*, extruded in plasmoptysis (according to FREY-WYSSLING 1940*a*).

cytoplasm and moving these by means of a magnetic field. As soon as the field is switched off, the particles return elastically to their original position (compare SEIFRIZ, 1942, 1945 a, b).

Especially interesting is the *spinning capacity* of the cytoplasm which is apparent from the fact that long strands can be drawn from it (SEIFRIZ and PLOWE, 1931). Often this phenomenon can also be observed during plasmolysis in the form of the so-called strands of HECHT (Fig. 100), although this name is scarcely justified, since their importance has already been pointed out by CHODAT (1907) many years before HECHT (1912). From Fig. 100a it is apparent that spherical boundaries as claimed by RHUMBLER (1898) occur only in a few fibres in a very imperfect form. A similar fact, which shows the non-liquid state of the cytoplasm is the "angular plasmolysis" of sea-urchin eggs (RUNNSTRÖM and MONNÉ, 1945; RUNNSTRÖM, MONNÉ and WICKLUND, 1946). In the plasmoptysis of Spirogyra cells the plasm can be drawn out into a long strand which contracts rhythmically (Fig. 100b). SEIFRIZ (1929) has shown that the cytoplasm of amphibious red globules can be drawn out to its three-fold length and the nucleus even up to 20 times its original length without the occurrence of any drops. All these properties of the cytoplasm are inconsistent with the hypothesis of a true liquid. They point rather to some fibrous submicroscopic structural element. If this conclusion is compared with the existence of the polypeptide chains ascertained by chemical means, it seems reasonable to attribute the microscopic fibrous and fibrillar structure observed in the living cytoplasm or after fixation to its submicroscopic framework of long chain molecules.

Even more than the formation of strings, the formation of layers in certain spermatocytes and spermatides (MONNÉ, 1940, 1941, 1942a) points to a gel-like structure of living matter.

Thus we have to deal with the paradox that the cytoplasm shows at the same time characteristics of liquids (fluidity) and of solid substances (elasticity). It is *solid and liquid at the same time* to an extent scarcely observed in any other colloid. The task of submicroscopic morphology consists, therefore, of drawing up a structural scheme which explains the double nature of the cytoplasm at the boundary of the two classical aggregate states. By doing so, we should gain more than by adhering to the cytoplasm either as a liquid or as a gel, neither of which can be true in a general sense.

Structural scheme of the cytoplasm. Fig. 101 gives a rough scheme of the molecular fine-structure of a strand of plasm. The members of the fibrous framework are extraordinarily thin; the order of magnitude of their thickness is that of the molecular cross-section of a single polypeptide chain. Their side chains are interconnected by junctions of varying nature. Thus an extremely fine network is formed, a ***molecular framework***. The meshes of this framework contain the interstitial substances: a solution of salts in water, and lipids, including phosphatides. However, the distribution of these compounds in the interstitial spaces is not random; they are arranged round the hydrophilic and lipophilic groups of the polypeptide chains which are not saturated by bridge bonds.

According to WRINCH (1941), fibrous plasm strands might be built up of globular protein molecules. This is in contradiction to the fibre anisotropy. Yet it is conceivable that the shape of the molecules of the plasm proteins is intermediate between the two extremes: fibre and globule (see Fig. 155, p. 219) and is perhaps capable of changing from the one shape into the other.

With this model (Fig. 101) all particulars of the cytoplasm properties can be understood. It should be stressed, however, that its object is not the elucidation of the phenomena of living matter but only *the establishment of morphological facts.*

The high *water content* of the cytoplasm (70 to 80 % or more) is caused by the considerable width of the meshes of the polypeptide framework. These are much larger than is apparent from our scheme, which must be regarded as a projection. The interstitial regions can be so spacious that no longer all water dipoles are fixed by the framework; some can move freely. In that case excretion of water from the cytoplasts and thus vacuolisation becomes possible. As a rule, however, all the water is somehow loosely bound by main chains or side chains and thus takes part in establishing the maximum state of swelling.

Fig. 101. Scheme of molecular plasm structure during flow; · = junctions.

At the transition of the protoplasm into its state of rest, the amount of water is gradually diminished by narrowing the meshes and perhaps partly replaced by lipids, as hydrophilic groups are screened off by phosphatides, sterines and the like. The determinant structure and the organisation of the framework which governs the processes of life can thus sometimes be preserved for years (spores, seeds). Evidently this natural dehydration with which the plasm rest sets in cannot be carried out artificially, since the change in the framework structure has to proceed step by step along with the dehydration caused by neutralising or screening the hydrophile groups, without changing those configurations of the molecular structure that are important for the maintenance of life.

The physical properties *fluidity*, *plasticity* and *elasticity* must be attributed to the character of the various junctions. The more these are dissolved by the processes described in the preceding chapter, the more liquid the cytoplasm becomes. However, the junctions must never be weakened all at a time; certain side bridges must always remain untouched, in the first place the main valency bonds. In other words, the cytoplasm may never become a true sol in which the polypeptide chains can move freely. Certain bonds always remain preserved and these cause the elastic properties. The dissolution of all junctions would result in the death of the cytoplasm by liquefaction.

We cannot sufficiently stress the fact that the various compounds in the cytoplasm must under all circumstances be united in some sort of pattern. The chemical plasm compounds which thus far have been isolated form by far the greatest part of the plasm substance and leave hardly room for any other, unknown, class of compounds which could play an important part; but the molecular structure of these units does by no means suffice to intervene directively with the processes of life. True, the asymmetric synthesis in organic chemistry has taught us, that these units are capable of specific achievements in as far as they possess asymmetric carbon atoms, in which case their presence results in optically active compounds and prevents

the formation of racemic mixtures. However, this is not yet a proof of life. In order to be able to direct the processes of life, the chemical structural units must be organised in a very complicated pattern. For here, as in the case of asymmetric synthesis, the theorem applies: *specific structures can be formed only by the agency of corresponding structures.* For this reason the chemical compounds of the cytoplasm would not be capable of accomplishing any purposeful work if, without definite position in space, moving freely, exposed to the arbitrariness of the Brownian movement like liquid colloid particles, they would move about at random in a sol[1]. In this context we may mention the newer results of enzym research from which it is apparent that in order to be effective, the active groups of the ferments must be attached to special carriers (compare MITTASCH, 1936). In the endoenzyms (BERSIN, 1939) this carrier is a part of the cytoplastic molecular framework. As long as this remains intact, the cell is alive; when it breaks down death sets in, while the enzyms retain their activity and start a wild destruction of the cytoplasm compounds, which is designated as *autolysis.*

The great marvel of the living molecular framework is its striking mobility which becomes apparent in plasm flow. In this flow the chains are orientated not only in small but even in microscopic regions, as indicated by the visible strand formation. The parallel alignment of the chains is often so pronounced that birefringence of flow occurs (ULLRICH, 1936a; amoeboid movement of the rhizopodiae, SCHMIDT 1937a, 1941b). The whole movement is only intelligible if a great number of junctions is continuously being formed to be broken down shortly afterwards. During flow, however, the most important constitutional compounds, above all the homopolar valency bonds must either remain intact or be continuously renewed. *The fundamental difference from dead gels lies in the fact that in the cytoplasm the junctions are continuously reconstructed.* The pattern of junctions of living matter is not rigid and fixed as, for instance, in gelatine- or still more in cellulose gels; its only permanent feature is its continual change!

The reconversion in the system of junctions proceeds according to some definite plan about which we remain completely in the dark. Anyhow a temporary change in stability can also be produced artificially owing to the thixotropic properties of the cytoplasm (see p. 46). By mechanical means (pressure, shock) a reversible liquefaction can be brought about. Such brutal interferences are always followed, however, by a temporary or lasting more or less serious damage to the cytoplasm (see p. 119).

As mysterious as the constant changes in the molecular framework is the fact that parts of cytoplasm separated from the protoplasm can vegetate further on their own hook. Thus, if life is confined to quite specific molecular configurations, i.e., structures of the molecular framework, all essential groupings have either to occur quite often in each cytoplast or can continually be renewed, in order to make it possible that separate cell fragments show the same phenomena of life.

[1] In the ordinary sense a liquid possesses no structure. It has been found, however, that also, in liquids certain mutual positions of the molecules have preference above others (BARTHOLOMÉ 1936) — even if these are not predestined to a regular order by a pronounced fibrous shape — so that in the future the *structure of liquids* will come more and more to the front. The cytologists who declared cytoplasm, nucleus and plastids to be liquids set value principally on the fact that these constituent parts of the protoplasm are *structureless* in as far as they are shown to be isotropic and optically empty. Now, to keep step with the progress of science, one might be tempted to consider the colloids carrying life as *structured* instead of structureless liquids. In doing so, however, we would not gain much, for the biocolloids have no structure in the ordinary physical sense, but are organised in an exceedingly complicated way. For this reason we ought to speak of "*organised*" liquids.

The *development* of the organism is presumably also governed by special specific groupings in the molecular framework, which can be designated as *morphogeneous configurations*. However, in contradistinction to the majority of active groups regulating the metabolic process, these configurations do not at all occur in every variety of cells; they are confined to the cells of certain tissues and probably located in the nuclei. A tissue of this kind acts as "organiser" (SPEMANN, 1936; WEISS, 1939; BALTZER, 1942) since the processes of development concerned can only take place in its presence. This organiser can be influenced by chemical means. LEHMANN (1937 a, b), for instance, has succeeded in controlling chorda formation by treating the gastrula of *Triton* or *Rana* with lithium chloride. This can be explained by assuming that the essential morphogeneous configuration of the molecular framework is changed either substantially by chemical compounds (for instance hydration) or only in its configuration in space (for instance by changes in the distance between decisive groups) in such a way that they can no longer fulfil their task. These morphogeneous groups often require hormones to be activated (HADORN, 1939).

Comparison with current opinions about the structure of the cytoplasm. The views about the submicroscopic structure of the cytoplasm developed in the first edition of this monograph have met with various criticisms. Before going into this criticism, we shall briefly discuss various points which make our theory fundamentally different from other ones.

It is not permissible to draw a parallel between "plasm viscosity" and the viscosity of liquids (compare Table XX). For here it is not merely a matter of friction between freely moving particles, but besides of an additional resistance offered by an elastic, amicroscopic framework. I completely agree with SCARTH (1927) when he writes that the fall of a particle through the cytoplasm is comparable to the zigzag path of hail shot falling through a brush heap, and that drastic methods like, for instance, centrifugation, forcibly destroy the fine framework of the plasm structure. The work of SCARTH also contains the essential points of this monograph in those places where he points out that the polarity and the capacity for growth of the cells are incompatible with the nature of a liquid such as that which has often been attributed to the cytoplasm and the nucleus.

Often microscopic strands are visible in the cytoplasm. As a dense, tough, "formed" plasm these are embedded in "unformed" plasm of semi liquid consistency. Such differentiations have been distinguished as kinoplasm and matrix (SCARTH, 1927), active plasm and paraplasm (v. MÖLLENDORFF, 1937a) or spongioplasm and enchylema (MONNÉ, 1942a). In some cases the two constituents can be separated in the centrifuge as plasm gel rich in lipids and a sol, poor in lipids but rich in mitochondriae, similarly as in the nucleus, where the nuclear framework and the karyolymph can be separated from each other. The microscopic string scaffolding or cytoskeleton (PETERS, 1937) is not to be identified with the submicroscopic structure. Undoubtedly the strands which are visible in the ordinary microscope originate from far-reaching bundling of the submicroscopic micellar strands postulated by us, but they certainly are not homogeneous and possess an invisible fine-structure consisting of proteins and lipids (MONNÉ, 1942a), whose elucidation belongs to the tasks of submicroscopic morphology. Similarly, it has to be found out whether the plasm liquor (enchylema, paraplasm, matrix) contains fibrous or spherical protein molecules (see p. 219).

A great number of the various hypotheses about the structure of the cytoplasm, discussed in former times (LUNDEGÅRDH, 1922, p. 242 and subsequent pages) are

irreconcilable with our own views. Nowadays the granular-, emulsion- and alveolar theories can no longer receive consideration. Taking clotted milk as an example, SEIFRIZ (1936) shows how the droplet theory takes account only of the relatively coarse units, whereas the fine-structure is caused by the fibre structure of the casein. He applies this model to the cytoplasm and is thus led to a scheme of the plasm structure which tallies well with ours, provided we omit the concept fibre and take into account that the living plasm does not represent a coagulum of protein particles which originally moved freely and independently of each other. However, the further comparison of the protein scaffolding of the plasm with a heap of rodlets seems less felicitous to me, since such a heap has a fortuitous, statistical character, whereas the structure of plasm must be of a wonderful coordination. Its framework cannot represent an unordered pile, but must possess an organised and well-defined structure.

According to our present knowledge all hypotheses about plasm structure which postulate distinct submicroscopic particles (granulae, droplets, alveoles, ultramicrons) must be discarded as being corpuscular theories. Thanks to its *framework structure* the cytoplasm possesses no dispersed phase in the sense of the classical theory of colloids: both the framework and the interstitial substance are continuous throughout the whole space available. For the same reason BÜTSCHLI's foam structure- or *honeycomb* theory must be left out of account in spite of its numerous merits, for a honeycomb cell is a closed disperse region in contradistinction to the dispersing medium which fills space continuously.

FLEMMING's fibrillar theory, on the contrary, conforms very well to the condition of complete mutual pervasion made probable in this monograph. Here again, however the fibrillar structure has to be transferred to micellar regions. In fact, in a three-dimensional network both the contours of the meshes and the meshes themselves fill all space continuously. MONNÉ (1946a) is of opinion that the plasmatic fibrils do not form a network, but are only entwisted to a braided work (in German: Flechtwerk). To my mind this depends on whether we have to do with a plasm gel or a plasm sol (p. 118). In the first case there must be some interaction between the invisible fibrils, whilst in the second case they may be independent from each other.

The fibrillar theory has been developed partly on the basis of fixed structures. This derivation is not as unapt as has often been suggested, since on fixation the molecular strings of the framework combine into coarser strings by *directed coagulation* and can thus become microscopically visible. It is only because the cytoplasm actually possesses the network structure assumed, that the good fixations relatively true to nature, attained by cytologic micro-technique, become comprehensible. In this process the molecular framework may shrink, be coarsened, deformed and disturbed by the disappearance of numerous junctions, but a sharp separation of coagulum and serum as in the case of *protein solutions* of like concentrations (milk, fibrinogen containing blood serum) does never occur.

The plasm framework of polypeptide chains which to a certain extent proves to be very stable with respect to hydrolysing substances might be identical with REINKE's plastin (1881). The latter represents the insoluble and not easily digestible part of the cytoplasm; both these properties belong to the high molecular cytoplastic protein framework. On drying, it becomes still less digestible, which might be connected with the fact that the molecular strings of the framework combine into coarser strings, as in fixation, and then are less accessible to the destructive ferments

of the metabolic process. This increased resistance against destructive action, known as *denaturation* of proteins, is again a result of the molecular structure of the framework; if the protein molecules were dissolved in the plasm, they ought to peptize again on adding water, like powdered milk, albumin, etc.

The introduction of *plastin* as a collective concept for the entire protein frame is very practical to describe these conditions. Although REINKE did not think of a molecular network, the properties of the polypeptide framework tally well with the characteristics given by him. The original concept "plastin" has no chemical meaning, for it is characterised only in the negative: insolubility, indigestibility, absence of phosphatides and lipids; in short: what remains if everything sensitive to mild physico-chemical intervention has been removed. The fact that often no plastin is left is no proof that a molecular framework does not exist, for, dependent on the special structural chemical conditions, this framework too can undergo hydrolysis. REINKE's expression plastin is therefore a *morphological concept* like chromatine in the nucleus, and as such is scarcely dispensable for purely descriptive purposes. For this reason it is regrettable that KIESEL (1930) after having isolated certain protein-like skeleton substances from the plastin of slime moulds (in REINKE's original sense) has applied the name "plastin" to a well-defined protein compound. It is better to give a new name to these chemically defined substances, and to maintain the plastin concept in its original *morphological* meaning proposed by the work of REINKE (1881), ZACHARIAS (1883), BERTHOLD (1886) and others.

Cytologic morphology needs collective concepts such as lignin, chromatine, lipids and plastin, which do not designate well-defined chemical compounds but classes of substances which are defined in a morphological sense as microscopic phenomena. If these concepts, created by the microscopist, are not satisfactory from a chemical point of view, chemistry has to provide a *new* and more suitable terminology. In fact, microscopic microchemistry, adjusted to morphology, can never satisfy the high demands of an exact chemical and structural description.

BENSLEY has succeeded in giving a closer characterisation of the structural proteins of the liver (1934a, 1938, 1943). The mobile proteins are soluble in 0.85 % NaCl. On further treatment with n/200 NH_4OH the mitochondriae and the nucleo-chromatin are dissolved. From the remainder a homogeneous substance, plasmosin, can be extracted with NaCl 10 %. This is described as a gel- and fibre forming constituent of the protoplasm (BENSLEY, 1938). The protein ellipsin is left, and BENSLEY compares it with REINKE's plastin. Plasmosin is compared with the muscular protein myosin (BENSLEY, 1943); according to MIRSKY and POLLISTER, however, it has its origin in the nucleus and should be regarded as a nucleoprotein.

Criticism of the theory of junctions. The submicroscopic reticular structure of the cytoplasm has been decidedly rejected by HÖFLER (1940). In his investigations on cap-plasmolysis (compare Fig. 105 and p. 124) he succeeded in making the cytoplasm of *Allium* cells swell up to 10 and more times its original volume with the aid of alkali salts, without causing the cells to die. HÖFLER concludes that no molecular frame work can be present, for the enormous swelling has pushed the polypeptide chains so far apart that they must completely change their mutual relations and positions. This reasoning would be correct if only heteropolar cohesive bonds were operative in the cytoplasm. It has been pointed out, however, that the molecular framework of the cytoplasm contains in addition other junctions, which are not sensitive to swelling. If only a few valency bonds exist between the chains, enormous degrees

of swelling (up to 30) can be attained without breaking down the molecular framework (see p. 48). It seems to me, therefore, that Höfler's interesting observations are in favour of the theory of junctions rather than against it, for what system other than a molecular frame could be inflated to a ten-fold volume without losing its inner structure? The fact that the latter has been preserved is proved by the possibility of returning to the normal state of swelling in which the plasm flow is resumed.

Fig. 102. Cytoplasm substance from spinach leaves in the electron microscope. Image scale 75000 : 1 (according to Menke 1940).

In spite of its magnitude, cap-plasmolysis must be designated as limited swelling, and in the case of colloids with limited swelling we always have to deal with a meshwork of long chain molecules.

Rod-shaped particles in chain-like arrangement have been observed in electron microscopic investigations on the cytoplasm substance of spinach leaves (Menke, 1940a). This proves the existence of submicroscopic strands in the cytoplasm, although Menke attaches more weight to the presence of single particles. The arrangement of the rodlets shows, however, that they are invisibly connected among each other.

Particularly interesting are the spherical single particles found by Menke, since they may represent globular protein macromolecules. It is, therefore, possible that the cytoplasm is a mixture of fibrillar and globular protein (Wrinch, 1941).

With particular care we have to take account of any objections of a physico-chemical nature, since these concern the fundamentals of the theory of junctions projected. According to Schulz (1939) the Van der Waals cohesive forces are too small to establish fixed bonds between molecules, so that a continual interchange of these junctions must be assumed. Considering the labile nature of the invisible plasm structures, it seems to me that this should be valued as constructive rather than destructive criticism. The decisive point is, that the cohesive forces between the macromolecules of the cytoplasm act as *structure forming* elements, as is clearly shown by the structure of the mesophases (p. 36). According to Bernal (1940) and Fankuchen (1941) these forces can cause chain molecules which are up to 150 Å apart to form oriented gel structures!

K. H. Meyer (1940a, p. 607), on the contrary, regards the cohesive bonds as true junctions. According to him, the distinction of four different types of junctions

goes too far; a division in cohesive and valency bonds would amply suffice. Against this objection it can be said that chain molecules with homopolar cohesive bonds (for instance waxes) or chiefly heteropolar cohesive bonds (for instance cellulose) show a fundamentally different behaviour in the physiological range of temperatures. Whereas wax becomes plastic at 37^0 as a result of the weakening of the homopolar cohesive bonds, a separation of the polysaccharide chains in cellulose can only be brought about by suitable hydration of the heteropolar cohesive bonds. Admittedly, homopolar cohesive bonds can also be solvated by lipophilic swelling media (benzene, etc.). Under physiological conditions, however, solvating media of this kind need not be considered, and it would seem that the division suggested suits the purpose in the case of living hydrogels. Similarly, the reaction to chemical interference (hydrolysis, hydrogenation, etc.) of a gel frame containing only heteropolar valency bonds would be fundamentally different from that of a gel whose chain molecules are connected by homopolar valency bonds.

We must take account, however, of another objection. In view of the principle of short range order, neighbouring chain molecules cannot cross in the manner indicated in the first edition. Rather will they be parallel to each other in parts as a result of cohesion. On account of the fact that in this range numerous junctions are active, K. H. MEYER introduced the notation of *regions of junctions* (Haftstelle instead of Haftpunkt). However, this improved terminology does not fundamentally affect the principles of the theory of junctions. The fact that the cytoplasm is no corpuscular dispersed sol but rather a colloid with a well-defined molecular structure remains of fundamental importance. The presence of fibrous structural elements can be considered as proved. Since we have no knowledge of the finer molecular structure, we must at the present moment content ourselves by saying that the cytoplasm structure consists of a strongly intertwined molecular framework of plastin strands or strings, to which lipids, phosphatides, sterines, inorganic ions and water molecules are attached.

Plasm flow. The touchstone for the correctness of any theory on plasm structure is the self-consistent explanation of plasm flow. For this reason the latest results of the investigations on this important phenomenon of life will be shortly discussed.

The cells and plasmodia, in which thus far the flow could be analysed more accurately, all show a sol-like liquid inner plasm (plasm sol) and a gel-like, solidified skin plasm (plasm gel) (LEWIS, 1942; MARSLAND, 1942; MOYER, 1942; SCARTH, 1942; SEIFRIZ, 1942; ANDRESEN, 1942). The difference in colloid state between the two types of plasm is demonstrated by the Brownian movement of the microsomes present. Whereas these are moving vividly in the bulk plasm (endoplasm) where the viscosity is low, they have the appearance of being frozen in the solid skin plasm (ectoplasm).

In cells with amoeboid movement plasm flow is maintained by continuous gel-sol transitions. The back part of the cell contracts, and simultaneously part of the gel-like ectoplasm is converted into liquid endoplasm. This can be observed directly, because microsomes enclosed in the plasm gel become mobile, show increased Brownian movement and finally are carried away by the bulk plasm. In the front part of the amoeba the skin becomes thin and is bulged outward as pseudopodium by the inner pressure. The entering stream of endoplasm solidifies into a gel at the side walls of the bulged part and thus rebuilds the skin at the same rate at which the amoeba moves forward. To explain plasm flow we need, therefore, a deeper understanding

both of the *contraction* and of the *gel-sol transition* of the protoplasm. The reversed phenomenon: solidification of the endoplasm into a gel has been observed in the fertilization of sea-urchin eggs (MIRSKY, 1936).

If a gel is liquefied under isothermic conditions, the volume can either increase (gelatin, agar), or remain constant (Na-oleate and other thixotropic gels), or decrease (methyl cellulose in water); compare FREUNDLICH (1937). A decrease in temperature or an increase in pressure favours gel formation in the first case and sol formation in the third. In protoplasm BROWN (1934) and MARSLAND (1942) have checked this experimentally with the aid of an ingenious apparatus, which allows of microscopically observing cytologic objects in a bomb provided with windows, at hydrostatic pressures up to 1000 at. Moreover, the bomb can be mounted in a centrifuge (compare p. 123) which makes it possible to investigate the motion of inclusions in protoplasm exposed to pressure. The experiments showed that the gel of the ectoplasm *liquefies* reversibly at high pressures.

In the liquefied cytoplasm all plasm flow has stopped, not only the creeping motion of amoeba cells but also the rotation in *Elodea* cells. Even the process of cell division in sea-urchin eggs, which show a beginning constriction, comes to an end. If the high pressure is not maintained too long, the cytoplasm re-solidifies into a gel at normal pressure, and plasm flow and cell division take their normal course again. These experiments show that the plasm sol is not capable of plasm flow and of constrictions such as those necessary for cell division, because no gel structure is present to support the formative forces.

LEWIS (1942) has shown that in the sol-gel transition the solidifying protoplasm is contracted. This seems to contradict the above statement that the plasm volume is increased on setting, since a hydrostatic pressure liquefies the gel. These volume changes are so small, however, that they cannot be observed; their existence can only be derived indirectly from the influence of the pressure on the cytoplasm. The contractions reported by LEWIS are no equilibrium reactions; the plasm is reduced in length and increases its thickness correspondingly. In the division of fibroplasts, for instance, the division of the nucleus is accompanied by the occurrence of a thickened ring of plasm gel, which divides the cytoplasm into two parts by contracting. This explains how the ectoplasm of the amoeba can exert a pressure on the bulk plasm by contracting.

It is of particular interest that these contractions take place *rhythmically*. With the aid of kinematographic (80 fold) quick motion pictures, SEIFRIZ (1937) has shown that the plasm flow is a *pulsatory* movement. KAMIYA (1940, 1942) succeeded in analysing the rhythmic flow of the cytoplasm in a plasmodium strand of *Physarum polycephalum* by means of variable one-sided counter pressures which exactly balance the flow. He observed complicated oscillatory changes in pressure, which can be resolved into pure sine oscillations by FOURIER analysis. This shows that the plasm flow of slime moulds is a polyrhythmic movement caused by numerous sine-like contractions of various periods.

This rhythmic contraction is reminiscent of the muscle activity, which is due to the contractibility of polypeptide chains (see p. 211). It is therefore likely that also the plasm flow is maintained by contractible protein chains in the cytoplasm. It is clear that the contractible polypeptide chains can unfold their full activity only in the plasm gel, although SEIFRIZ (1942) claims that also the "liquid" cytoplasm (down to relative viscosities of about 2) is contractible. It would seem that these statements

once and for all refute the idea that the cytoplasm consists of a liquid with freely moving particles.

Cell polarity. Another important fact which has to be explained by a consistent theory of plasm structure is the polarity of cytoplasm. This property is especially evident with the eggs of *Echinodermata* and *Amphibia.* These cells show a definite animal and an opposite vegetative pole. Sometimes the animal pole is characterized by a papilla, but this is not necessary. There is as well an invisible physiological polarity. Were the structural elements of cytoplasm independent of each other as in a liquid, no fixed polar arrangement within the cytoplasm would be conceivable. The polarity, therefore, must be inherent to the plasm gel. As the cortex of the egg has undoubtedly a gel-like character and in this state is capable of considerable active transformation when the fertilization membrane is formed (RUNNSTRÖM, 1944), one might be inclined to attribute the polar properties to this cortical layer. But MONNÉ (1946b) finds, that there is a dorsoventral gradient also within the egg, the animal cytoplasm being more solidified and the vegetative cytoplasm more liquefied. It is admitted, that the heteropolar organisation is predetermined by the foregoing cell division (LEHMANN, 1945). Cytoplasmic currents do not destroy the heteropolar organisation. From this fact I suppose that important junctions of the plasmatic framework are still existent throughout the moving cytoplasm. As MONNÉ points out cytolysis of the sea-urchin egg is preceded by violent protoplasmic currents. This increased movement is due to a complete liquefying of the cytoplasm which is followed by the desorganisation and the death of the cell. Complete desintegration of the junctions, therefore, will never occur in living cells.

c. *De-mixing of the Cytoplasm*

Separation into different phases. As long as there exists a certain equilibrium between the living molecular framework with its free lipophilic and hydrophilic side groups on the one hand and the amount of lipids and water on the other hand, the cytoplasm remains microscopically homogeneous, hyaline, as clear as water and optically empty. Also in the physico-chemical sense we have to deal with a homogeneous system, a pseudophase (p. 48) without inner surfaces. This system is bound to demix, if one of the three components: protein, lipid or solution, increases in quantity to such an extent that the state of mutual equilibrium can no longer be maintained, and similar molecules cluster together and are separated from the rest of the cytoplasm by a phase boundary.

Formation of vacuoles. GUILLIERMOND (1933) attributes the origin of vacuoles to the formation of hydrophilic colloids in the cytoplasm. These colloids attract water, are hydrated and thus cause demixing. It is quite possible that the vacuoles are formed in this manner. Clearly, besides colloids, also salts, which accumulate in the cell as a result of the continuous absorption of matter, may initiate the accumulation of water in some of the meshes of the molecular framework. Then, according to the laws of surface tension, the aqueous phase assumes a spherical shape and pushes the molecular or micellar framework aside. It may therefore be assumed that the framework of the cytoplasm has a higher density in the neighbourhood of a vacuole. Thereupon lipids are accumulated in the boundary layer (compare Fig. 106).

The colloid content of the vacuolar liquid can be proved or at least made probable in several ways. The viscosity, for instance, is about twice that of water (WEBER, 1921; PEKAREK, 1933) or of aqueous solutions with the same salt content

as the vacuoles (compare Table XX). Particularly suitable are measurements according to the fall method in the large terminal vesicle of *Closterium* algae, in which the sedimentation of gypsum crystals can be measured accurately (FREY, 1926c). From STOKES' law one derives a relative viscosity of about 2.5 for the cell sap. The experiment shows, moreover, that the boundary of the vacuole is not a smooth surface, for a number of crystals do not follow the shortest path, but glide down

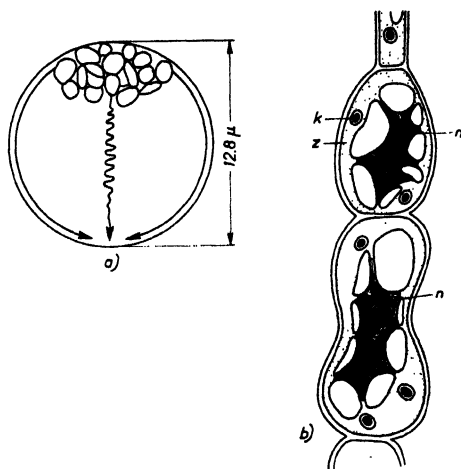



Fig. 103. Vacuoles. *a*) Sedimentation of gypsum crystals in terminal vacuoles of *Closterium* (according to FREY 1926*c*); *b*) pathologic giant cells of *Aspergillus niger* fixed with FLEMMING. Cytoplasm z and nucleus k have not changed much; in the cell sap, however, a voluminous precipitate is formed (according to FREY 1927*a*).

along the wall (Fig. 103a). For this reason, when measuring the time of fall of crystals transversing the cell sap, one must always be observant of the time needed to detach the particle from the phase boundary (WEBER, 1921).

In certain cases the cell sap solidifies on fixation, as represented in Fig. 103b in the case of pathological giant cells of the fungus *Aspergillus niger* (FREY, 1927a). Here the difference between the colloid systems of the cell sap and the plasm is evident. In the cytoplasm the framework structure prevents a separation of the different components, whereas in the cell sap demixing occurs. The coagulated vacuole of Fig. 103b betrays a coarse structure of fibrous, entangled bodies. From this we may conclude that the colloids in the cell sap do not possess a structure comparable with the cytoplasm, but represent sols with movable particles without definite mutual positions. Here coagulation actually results in an orderless "pile", indicating an unordered state before the precipitation. The end groups of the organic compounds which are the constituents of vacuolar colloids are not screened off as in the plasm and are consequently reactive. This is made use of in the *vital staining* of the vacuoles. Their colloids, which evidently carry acid groups, are usually readily coloured by basic dyes. In the cytoplasm, the cell nucleus (BECKER, 1936) and the living, still growing cell wall, on the contrary, vital staining is much less easily obtained. According to STRUGGER's investigations (1935/1936) in vital staining the p_H of the surrounding liquid is the main factor in dyeing; this is true not only in the living state, but according to PISCHINGER (1937), DRAWERT (1937b) and others also in fixed protoplasts. In the sense of the theory of junctions this means that the acid and

basic groups of the molecular framework, which are screened off in the I. E. P., must first be liberated by slight hydrolysis in order to be capable of reacting with the dyestuff.

The vacuoles owe their existence to substances which are temporarily or definitively excluded from interaction with the molecular framework of the cytoplasm. For this reason these sap-filled spaces represent places in which excretory (definitive elimination) or reserve substances (temporary elimination) are stored. All cell sap components like anthocyanins, tannins, glucosides, etc. must therefore be regarded as substances eliminated from the cytoplasm. Hence the vacuoles are primarily excretory organelles in which all kinds of substances which are inconsistent with the cytoplastic molecular structure are stored; their function of regulating osmotic phenomena is only a secondary task.

Lipidic drops. Similarly as in the case of water there is an upper limit to the amount of molecularly dispersed lipids bound by the cytoplasm structure. Beyond this limit the lipid molecules cluster together into globules which represent an analogy to the vacuoles; they might be called lipidic vacuoles as counterpart to the aqueous vacuoles. Apart from the surface films at the phase boundaries, as a rule neither the lipidic drops nor the vacuoles possess a structure. Their content is semi-solid to liquid, optically isotropic and homogeneous in the physico-chemical sense.

These regions, which are homogeneous and therefore foreign to the plasm, are usually regarded as reserves for the metabolic process. In this connection we think in the first place of oil and fat containing seeds, which mobilize their lipids during the germination. However, we find also lipidic secretions of an irreversible nature, which can scarcely be considered as reserve substance (fatty degeneration, lipophanerosis).

Aleurone grains. The accumulation of proteins in the cytoplasm leads to two different kinds of demixing. On the one hand, easily soluble proteins with globular molecules of relatively low molecular weight may accumulate in the vacuoles of storage cells, where they crystallize or solidify into *aleurone grains*. However, if the amount of high-molecular protein chains in the cytoplasm increases and these chains cluster together, plasmatic fibrils are formed (KÜSTER, 1934a, 1935a). In other words, the morphological properties observed depend upon whether reserve proteins or plasm proteins are demixed. Originally the aleurone grains are liquid vacuoles, which lose water by active dehydration. In this process the various vacuole components precipitate according to their solubility. In the aleurone vacuole of *Ricinus* seed, for instance, the almost insoluble magnesium-potassium salt of inositol phosphoric acid (phytin) is precipitated first as a body called "globoid". Thereupon the reserve proteins which in contrast to the insoluble skeletal proteins are corpuscularly dispersed, begin to arrange themselves into the lattice order of a crystalloid (compare p. 216) and to fill the available space. Finally also the last remnants of liquid, containing an easily soluble albumin, solidify into a homogeneous substance surrounding both globoid and crystalloid. On mobilization of the reserve substances the dissolution proceeds in the reversed order: the albumin is dissolved first, thereupon follow the protein crystalloid and finally the mineral globoid.

Origin of fibrils. Formerly the formation of contractile fibrils (Protozoa), and of muscular fibres (Metazoa), was regarded as an extremely astonishing achievement of the cytoplasm. Nowadays, however, this kind of differentiation can be understood from a morphological point of view, since the framework structure of the cytoplasm

itself consists of polypeptide strands. These structural elements need only be accumulated and ordered to a certain extent to produce fibrillar plasm structures. However, the mechanism of *contraction* of these fibrils remains a secret (compare p. 211).

Phase separation by centrifuging. The phases brought about by demixing can be separated in the cell by centrifugal forces. Here the centrifuge microscope of NEWTON HARVEY (1930) renders special service. Fig. 104 shows a centrifuged sea-urchin egg of *Arbacia punctulata*. Centrifuging has elongated the egg cell and its various components: pigment grains, yolk globules, chondriosomes and oil droplets appear neatly separated. Optically homogeneous plasm, containing the nucleus, accumulates in the less dense part of the cell. The striking layer formation seems to indicate a stratification phenomenon in a liquid. This, however, is contradicted by the following interesting and extremely remarkable fact: by further centrifuging, the egg cell can be separated into two halves, as indicated in Fig. 104 by a line. In this process a clear part containing the nucleus and a pigmented part without nucleus are formed. Both can be inseminated and are then capable of division (E. B. HARVEY, 1933), and the part which does not contain the nucleus may sometimes be induced to division without any nucleus. E. B. HARVEY (1936) concludes from this: "It must therefore be the "ground substance" which is the material for development — the matrix which is not moved by centrifugal force and which, in the living egg, is optically empty". LEHMANN (1945) points out that in the outer layers of the *Tubifex* and the sea-urchin egg, there must be a morphogenetic pattern, which cannot be destroyed by centrifugal forces.

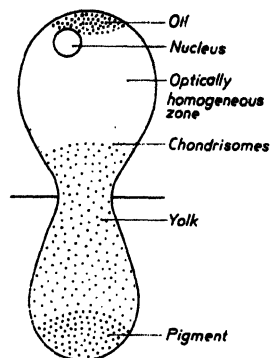



Fig. 104. Egg cell of *Arbacia punctulata* after centrifuging (according to E. E. HARVEY 1936)

In other words, the method of centrifuging also leads to the conclusion that an invisible ground framework must exist, which is torn apart in the centrifuge by the oil droplets, yolk and pigment particles respectively, as a result of their different weights. The microscopically visible particles have to move in the opposite direction through the meshes of this molecular framework without damaging it seriously, since division and growth of the plasm fragments separated by centrifugation still takes place afterwards. For this reason the framework must either possess very coarse meshes or else it must be possible for the important molecular groupings, whose mutual positions have been altered by centrifugation, to be restored to their original arrangements.

By centrifuging the invisible plasm frame is orientated, for the drawn-out plasm neck shows positive birefringence with respect to the axis (PFEIFFER, 1941 b). Its reticular structure must possess an unexpected mechanical stability, for *Ascaris* eggs can stand centrifugal fields of 950 000 times gravity for 10 hours or 400 000 times gravity for 10 days (BEAMS, 1943), without expiring or losing their normal capacity for development, although, with the exception of the nucleus, all components of the cell appear to be completely separated from the cytoplasm. Nor can the polarity of *Tubifex* eggs be reversed by centrifuging (LEHMANN, 1940).

We must mention in particular, that neither the oil droplets nor the yolk and pigment combine into a homogeneous phase, but remain dispersed. This indicates the existence of surface layers which, either by their structure or by their electric charge, offer resistance against fusion with the neighbouring particles. It is quite

possible that the properties of the ground substance in which they are still embedded prevent the droplets from clustering together as might be expected from the laws of surface tension.

Separation of phases as a result of freezing. When freezing the cytoplasm, ice crystals are formed which are embedded in the dehydrated gel. Thus we get demixing by crystallization. According to LUYET (1939) the dehydration of the living hydrogel proceeds step by step. As long as the freezing is confined to excess water, such as that contained in the vacuoles of plant cells or coming from the metabolic process, the cell does not expire. It is only when the imbibition water which takes up the plasm structure is withdrawn from the living hydrogel, that the structure breaks down, and death of the cell sets in. The resistance of the plasm to low temperature depends, therefore, on its persistency to retain its hydration water and to safeguard this water against crystallization.

The crystallization of the imbition water, which is enclosed in the submicroscopic gel meshes and bound by hydration forces, can be prevented if the gel is cooled down to very low temperatures by rapid abduction of heat (thin layer!). This leads to a state which has been designated as *vitrification* (LUYET, 1937). The water molecules become immobile to such an extent that they cannot arrange themselves into a crystal lattice and retain their original positions with respect to the submicroscopic gel strands. In this way it is possible to preserve the "life structure" of thin plasm films for an arbitrary space of time, for instance in liquid air. However, the difficulty to induce such a "plasm glass" to resume its life functions consists of the fact that with increasing temperature the preparation must pass through the critical temperature range in which the water demixes from the gel by crystallising. The clear gel suddenly becomes turbid at about — 15° C and then the structural breakdown sets in, which normally causes death at slow cooling.

This phenomenon should not be mixed up with the wellknown fact that frozen plants can often be kept alive if thawed slowly. In these objects the imbibition water, indispensable to life, has not yet crystallised and one must merely take care that the plasm structure is not inundated and damaged by water melting too suddenly (water of the vacuoles and from the metabolic process).

d. *Morphological Principles of the Permeability Problem*

Like all physiological questions, the problem of physiological permeability is founded on morphological assumptions. The *lipid theory* of OVERTON (1899), the *ultrafilter theory* of RUHLAND (1912), the *mosaic theory* of NATHANSON (1904) and the modern, combined *lipid filter theory* of COLLANDER (1932, 1937a) are all based on certain morphological concepts which, it is true, have not been gained directly but via physiological experiments or reasoning (DAVSON and DANIELLI; 1943). Before going into these questions of the submicroscopic structure of cytoplast boundaries, a more accurate microscopic description of the cell boundaries must be given.

Fig. 105. Cell with cap-plasmolysis to demonstrate the various types of permeability (according to HÖFLER). *a*) Membrane permeability (p plasmolysis forecourt); *b*) intrability (z cytoplasm); *c*) permeability (v vacuole).

Problem of the boundary layers. The phenomenon of the cap-plasmolysis (German: Kappen-Plasmolyse) proves that certain plasmolytic agents are capable of penetrating into the cytoplasm, though not into the cell vacuole. For this reason HÖFLER (1931) distinguished between *permeability*, i.e., the passage from the outside through the cytoplasm into the cell sap, and *intrability*, in which only the plasm is reached. In addition one must in certain cases take into account a *membrane permeability*, i.e., a resistance of the cell wall to penetration (Fig. 105).

In cellulosic cell walls the membrane permeability can be neglected; they are permeable to all plasmolytic agents and therefore also to nutritious matter. Cutinised cell walls show a different behaviour (moss leaves, fern anulus, seed-coats); they are semipermeable to sugars. Any substance which has passed the cell wall reaches a second permeability resistance at the plasm surface. In former times it was assumed that all plasmolytic agents are retained at the plasm surface and there exert their plasmolysing action. This led to the paradox that, for instance, cane sugar, one of the most important amongst nutritious matter, could not penetrate into the cell. However, salts like KCNS, which cause the cytoplasm to swell and bring about cap-plasmolysis of the cell (see Fig. 105), have overturned this dogma, and nowadays it is supposed with HÖFLER (1934) that the main resistance in plasmolysis should be sought in the vacuole boundary, the so-called tonoplast, instead of in the plasm surface. This removes the contradiction that important nutritious matter should not penetrate into the plasm or, like KNO_3, should only do so with great difficulty.

This penetration into the cytoplasm falls under the concept of intrability. In the case of substances which cause no visible change in the plasm, their presence within the cytoplasm cannot always be proved easily. Yet the phenomenon can be very well observed, in the case of vital staining with chrysoidine, which often does not enter the vacuole. On the strength of these experiments it is supposed that the outer boundary layer of the cytoplasm is different in nature from the inner one around the vacuole.

In the phenomenon of deplasmolysis the plasmolysing agent must gradually invade the vacuole also. For this process HÖFLER wants to reserve the designation permeability. However suitable this distinction may be for botanical objects, in which most permeability studies have been carried out with the aid of deplasmolysis, it is inappropriate for animal cells, which do not possess vacuoles. I do not believe that HÖFLER's terminology which we want to apply in this context would cause confusion, since for cells without vacuoles intrability and permeability are, of course, identical. All the same we have to deal with a logical difficulty, for henceforward by permeability zoologists will understand *entrance* into the cytoplasm, whereas botanists will understand this as *traversing* the cytoplasm, i.e. entrance followed by excretion. If this excretion represents a passive diffusion, which is probably the case in deplasmolysis experiments, the difficulty is not of a fundamental nature. In most cases, however, where the excretion occurs in connection with the *natural intake* of a substance, energy is involved, and the phenomenon should then be considered as *active excretion* (*adenoid activity* according to OVERTON, see COLLANDER and HOLMSTRÖM, 1937). This does not apply to permeability investigations which are restricted to diffusion studies (BÄRLUND, 1929; ULLRICH, 1934; HOFMEISTER, 1935; MARKLUND, 1936), in which the concentration gradient applied is the only potential and no account need be taken of energy produced by the cell. Accordingly, the investigations connected with the respiratory sorption of substances (STEWARD, 1932, 1933; LUNDEGÅRDH and BURSTRÖM, 1933, 1935; HOAGLAND and BROYER, 1936; ARISZ and VAN DIJK, 1939; REINDERS, 1940; BRAUNER 1943) are not considered as permeability studies.

All permeability theories have in common, that the resistance to diffusion is located in the so-called plasmalemma or plasm membrane, which is the outer boundary layer of the cytoplasm and which is supposed to be either a submicroscopic lipidic layer, an ultrafilter or a combination of both these structures. One has never suc-

ceeded in making this lemma visible as an individual layer. Similarly, the hyaline ectoplasm of amoebae cannot be regarded as a permanent structure, since in amoeboid motion it can temporarily change into granular endoplasm. Non-the-less the hypothetic skin must be present, for microinjection experiments (CHAMBERS, 1928) show that dyestuffs, whose entrance is opposed by the surface, readily spread into the bulk of the cytoplasm. COLLANDER (1937b) regards this outer plasm skin as a lipid film free of proteins, and according to DANIELLI (1936) and TÖRNÄVÄ (1939) it consists of only two to four molecular layers, since, on increasing the surface by endosmosis, semipermeability of certain cells suddenly disappears at a certain surface size, and the cytoplasm begins to "leak". CURTIS (1936), on the contrary, has found with red globules that the semipermeable skin does not become "thinner" when stretched, but is continuously repaired by material supplied by inner layers. Probably, therefore, the plasmalemma does not represent a definite skin, but only a boundary layer in which lipids accumulate. Sometimes this accumulation comprises not only the plasmalemma but also visible plasm layers, so that the presence of lipids cause a distinct double refraction (MONROY, 1946).

Fig. 106. Scheme of submicroscopic plasm boundary in vegetable cells (according to SCARTH 1942). Lipids dotted. w = cell wall; e = hyaline ectoplasm, coated with plasmalemma; pg = plasm gel of endoplasm; ps = plasm sol of endoplasm; k = kinoplasm; t = tonoplast; v = vacuole; s = transvacuolar plasm strand; m = myelin tube; p = plastid.

According to NEWTON HARVEY (1937) the cell surface is *elastic*; this, according to model experiments, applies only to the surface of solutions containing proteins, whereas lipidic drops of lecithin (HARVEY and DANIELLI, 1936) or of oil in living cells (NEWTON HARVEY, 1937) possess no surface elasticity! It follows from this that proteins take part in the construction of the semipermeable plasmalemma, as I have already pointed out in earlier work (1935a, p. 144). Undoubtedly the elastic properties of the cell surface is determined by the network of protein chains. The scheme with *spherical, dispersed* protein molecules, which DANIELLI and HARVEY (1935) believe to apply to the structure of the phase boundary between oil inclusions and hydrophilic cytoplasm, can only hold good for surfaces without elasticity; the elastic plasmalemma rather possesses a structure with polypeptide chains (Fig. 107).

Probably the protein framework of the cytoplasm is built more densily in the outer layers and changes gradually into a much looser structure toward the inside. Accordingly, the cytoplasm in the egg of the sea-urchin is liquid, and a similar perspicuous difference in organisation between boundary plasm and inner plasm seems to exist in rhodophyta (HÖFLER, 1936b). At the phase boundary around the vacuole the greater density of the molecular framework and the accumulation of lipids must occur again, causing a renewed resistance against diffusion in this region.

SCARTH (1942) has completed and improved the scheme of the fine-structure of the plasm layers of plant cells suggested by me in the first edition of this monograph (Fig. 106). Underneath the cell wall lies the hyaline ectoplasm; its outer boundary is formed by the plasmalemma rich in lipids. The endo- or inner plasm consists at its periphery of plasm gel, with a network of polypeptide chains, and the

central part of plasm sol with more or less loosened junctions. It is intersected by strands of higher density which, as kinoplasm, connect the ectoplasm with the tonoplast.

Submicroscopic morphology of selectively permeable membranes. A clear picture of the permeation phenomena in the plasmalemma is obtained with the aid of the new permeability theory of K. H. MEYER (1935) and T. TEORELL (1935). This theory has

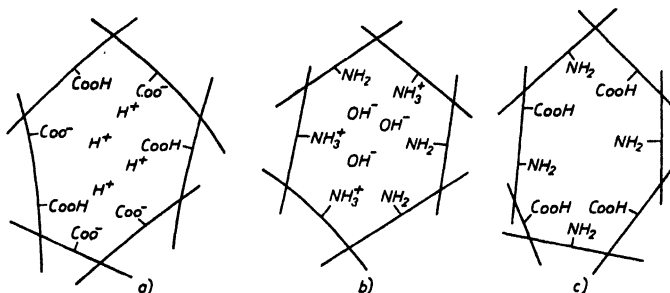



Fig. 107. Morphological principle of K. H. MEYER's and T. TEORELL's permeability theory (1935). Molecular frame *a*) anionic, *b*) kationic; *c*) amphoteric.

been developed for membranes with a framework as structure and for this reason is also suitable to the cytoplasm, which in our opinion is built according to a similar structural principle. The starting point of these new ideas is, that a molecular framework represents a gigantic, polyvalent and immobile kation or anion. In the case of the cytoplasm with its amphoteric character, the framework can act either as kation or as anion, according as the p_H changes (Fig. 107).

One should imagine that in the meshes of the framework, carboxyl groups or amino groups, or both, are fixed as immobile members of the main valency chains. The first case may, for instance, be realised in the pectin gel (BONNER, 1936a; DEUEL, 1943; 1947) of polyuronic acid chains (Fig. 107a), when the framework acts as an acid; the hydrogen ions are partly split off by dissociation and for this reason kations can diffuse more easily through this molecular structure than anions. Conversily, if the framework consists of basic chains (for instance chains of diamino acids, Fig. 107b), the anion permeability comes to the fore. Finally, the amphoteric cytoplasm (Fig. 107c) is more permeable to anions at low p_H and to kations at higher p_H values. This theory of the submicroscopic structure of the plasm surface and the cytoplasm may seem one-sided in that it takes into account only the ultrafilter action (ULLRICH, 1936b); yet lipid solubility is also included, if one realises that the molecular framework, especially in its outer regions, contains lipids and phosphatide molecules which are located within the meshes. WILBRANDT (1935) therefore rightly remarks that no sharp distinction can be made between the effects of filter action and solubility.

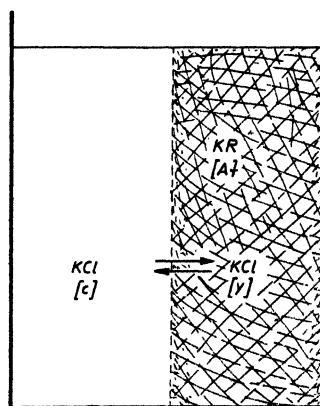



Fig. 108. DONNAN equilibrium between a molecular framework R with anionic dissociative groups (A) and a solution of KCl; (c) and (y) are the outer and inner equilibrium concentrations.

A colloid framework in the form of a polyvalent immobile ion, which is in contact with a true solution, represents a DONNAN system, even though no semi-permeable wall is present. For, as required for a Donnan equilibrium, the migration of the colloid framework into the surrounding solution is impossible, while its mobile ions can freely do so (Fig. 108). This consideration makes a theory of selective permeability possible.

Suppose an anionic, molecular framework R in the form of a potassium salt KR is in contact with a KCl-solution. Let A be the number of dissociation points of the framework anion, i.e., the concentration of the potassium capable of dissociation, y the concentration of the KCl penetrated into the meshes of the framework, and c the KCl-concentration of the outer solution. Then the ion product $[K] \cdot [Cl]$ equals $(y + A)y$ in, and c^2 outside the framework. Accordingly one obtains DONNAN's law[1]: $(y + A)y = c^2$.

TABLE XXI

DONNAN EQUILIBRIUM IN THE MOLECULAR FRAMEWORK

KR (A)	KCl total (c + y)	KCl inside (y)	KCl outside (c)
0.01	1.00	0.497	0.503
0.1	1.00	0.476	0.524
1	1.00	0.333	0.667
10	1.00	0.083	0.917
100	1.00	0.0098	0.990

DONNAN's exchange mechanism applies therefore to our framework structures, since the immobile anion R expels the mobile anion Cl from the meshes of the framework. As follows from table XXI, the Cl-concentration, y, in the framework decreases rapidly with increasing A. Thus, in order to establish DONNAN equilibria in the cytoplasm, no semipermeable membranes are required: the plasm as a whole acts as a gigantic, immobile and polyvalent colloid ion.

Now K. H. MEYER combines this result with the velocity of ion migration in a membrane possessing framework structure, in order to arrive at a quantitative expression for the permeation. Let U_K be the ion mobility of the kation and U_A that of the anion of the salt; further n_K the number of kations and n_A the number of anions of the migrated salt, defining these numbers in such a way that always $n_K + n_A = 1$.

Since the number of migrated ions is not only proportional to U but also proportional to the ion concentration in the molecular framework (compare Fig. 108), we have:

$$\frac{n_K}{n_A} = \frac{U_K(y + A)}{U_A \cdot y}$$

Since $n_K + n_A = 1$, n_K and n_A could be calculated if A and y were known. This, however, is not the case and for this reason the known outer concentration, c, is introduced. We have

$$y = \sqrt{c^2 + A^2/4} - A/2$$

and therefore:

$$\frac{n_K}{n_A} = \frac{U_K\left(\sqrt{4c^2 + A^2} + A\right)}{U_A\left(\sqrt{4c^2 + A^2} - A\right)} = \frac{U_K}{U_A} \cdot X.$$

[1] Usually the equilibrium is formulated in a more complicated way (HÖBER, 1922, p. 219): $(KCl - y)/y = (KR + KCl)/KCl$. In this less surveyable form $KCl = c + y$ and $KR = A$, which gives the above formula.

This relation is K. H. MEYER's starting point in his investigations on permeability. The ratio n_K/n_A can be determined potentiometrically. On the contrary, the ratio U_K/U_A and the factor X are unknown.

By carrying out measurements at different concentrations c, one obtains several equations from which both unknown quantities can be derived. Accordingly, the quantity A which MEYER designates as *selectivity constant* can be determined, and thus an important property of the framework can be expressed numerically.

For instance, from the well-known potential measurements of the apple skin by LOEB and BEUTNER (1912/1913), a selectivity constant A = 0.08 is calculated, i.e., the normality of the immobile framework anion equals 0.08 n.

MEYER has proved the validity of his theory in numerous synthetic and natural membranes. Undoubtedly it may therefore also be applied to the cytoplasm. To this end, however, we must take into account not only the ion mobility but also the lipid solubility. This is done by introducing the distribution coefficients of the migrating substance between membrane framework and outer liquid. If l_K and l_A are the distribution coefficients of the kations and anions respectively, the DONNAN relation runs

$$\frac{(y + A)y}{l_K l_A} = c^2,$$

since the concentrations of the ions in the framework are increased or decreased according as the distribution coefficients are larger or smaller than 1. The general permeability formula then takes the form

$$\frac{n_K}{n_A} = \frac{U_K\left(\sqrt{4c^2 l_K l_A + A^2} + A\right)}{U_A\left(\sqrt{4c^2 l_K l_A + A^2} - A\right)}$$

Although this formula has as yet hardly been applied to cytoplasmic permeability, I think it worthy of attention, since to a certain extent it enables a synthesis of the theories of permeability in biology. Each of the quantities occurring in it refers to a different principle of the usual theories of permeability. The ion mobility U is a measure for the filter resistance. In a hydrophilic framework with wide meshes, U_K and U_A would be equal to the ion migration velocities in water. By narrowing of the meshes, however, larger organic ions are impeded; and the filter effect will influence the quantities U. The effect of the solubility, in the first place the lipid solubility in the cytoplasm, is accounted for by the distribution coefficients l. The concentration gradient applied is expressed by c and the selectivity constant A is related to the electric phenomena accompanying the permeation. If the framework of a membrane has a negative charge, i.e., if it behaves like an anion, A becomes positive; in the reversed case, i.e., with a positively charged framework, A is negative. For the amphoteric cytoplasm the selectivity constant A, must therefore be either positive or negative dependent on the p_H of the nutrient.

If the p_H value of the imbibing liquid lies above the isoelectric state of the molecular framework, the cytoplasm behaves like an anion and thus is permeable to kations. In this state, weakly basic substances like amides (urea, methyl urea, malonic amide, etc.) will permeate more easily than at a p_H value below the I. E. P. Consequently, if one wants to distinguish amidophilic and amidophobic, or *urea-permeable* and *glycerol-permeable* protoplasts, the I. E. P. and the p_H of the cytoplasm concerned should be known. Otherwise it cannot be decided, whether the differences observed are properties common to the protoplasm as HÖFLER (1936a, 1942) believes, or whether they have been induced by the momentary state of the amphoteric cytoplasmic framework (BOGEN, 1938; ROTTENBURG, 1943). It may be assumed that the relation between p_H and I. E. P. plays a decisive part in the comparative permeability ex-

periments, so that in the end, like vital staining, they only represent new methods to determine the state of ionisation of the amphoteric cytoplasmic framework.

In the isoelectric state, i.e., in the case of a neutral framework, A = 0. Then the permeability formula reduces to $n_K/n_A = U_K/U_A$. In this state, therefore, the cytoplasm is no longer selective in its permeability to kations or anions.

Since K. H. MEYER's theory is based on potentiometry, it allows only of studying

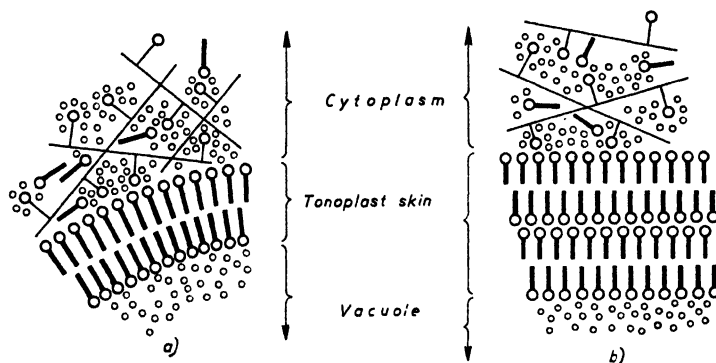



Fig. 109. Scheme of the submicroscopic structure of the tonoplast membrane, consisting of polar lipid molecules (compare Fig. 106). Hydrophilic groups white, lipid chains black, water molecules small circles. *a*) bimolecular; *b*) polymolecular film.

the ion permeability, which is of greater importance for the metabolism than the permeation of non-electrolytes studied so often with plant cells. For the time being, however, its application to cytoplasmic permeability is difficult (MEYER and BERNFELD, 1946), since of the many quantities which have to be accounted for, only very few are known in the cytoplasm. Nevertheless, the morphological principles of the considerations presented will doubtless bear fruit in future theories of permeability.

The tonoplast. Whereas the plasmalemma in plant cells probably differs from the inner plasm only by a protein framework of lower density and a corresponding greater lipid content, the vacuole skin or the so-called *tonoplast membrane* must possess an essentially different structure. It is this skin which impedes the entrance of hydrophilic substances into the vacuole and on the contrary strongly furthers the passage of lipophilic substances (PLOWE, 1931). It must therefore contain large quantities of lipids. Although this statement should not be generalised without further criticism, it certainly applies to many cases and especially to the classic example of *Allium* epidermic cells. In an interesting controversy WEBER (1932) and HÖFLER (1932) discussed the question, whether this lipid layer should be regarded as belonging to the cytoplasm or as membrane of the vacuole. From the point of view of molecular morphology this point of contention can be decided in the following way (Fig. 109).

As a result of the accumulation of lipids, the latter are no longer in equilibrium with the protein framework. Their molecular forces cause them to arrange themselves, turning their hydrophilic poles towards the hydrophilic inner plasm, the lipophilic ones towards the vacuole. As ascertained in the case of *Allium* (Fig. 46), the inner part of the vacuole consists of a hydrophilic liquid; the outer boundary, on the contrary, has a more lipophilic nature. In comparison with the cytoplasm, therefore, the lipid molecules in the vacuole must be arranged in exactly the reversed order.

The result is, that the boundary region of plasm and vacuole consists of a lipid layer which on either side, without sharp transition, gradually changes into hydrophilic regions. The boundary membrane will therefore consist of molecular double layers.

It is evidently difficult to say which part of this lipid layer belongs to the cytoplasm and which to the vacuole surface. The only criterion would be to determine to what extent the cytoplasmic protein framework penetrates into this layer. Since, however, this cannot be decided by vital staining, we must content ourselves with the fact that the boundary between the two cytological parts cannot be accurately determined. If, after destroying the cytoplasm, the tonoplast is pressed out of the cell, the thickness of the lipid layer clinging to the vacuole will depend on the previous treatment. Life, however, cannot be attributed to this structure, although it can remain in existence for days and can show osmotic changes in volume. Similarly, in the unimpaired cell the regulation of permeability by this layer in the usual permeation experiments is no sign of life, but a purely passive result of its lipid nature.

e. *Biosomes*

Chondriosomes or mitochondria are microscopic particles within the cytoplasm. They represent a special system in the cell which is designated as *chondriome* (GUILLIERMOND, MANGENOT and PLANTEFOL, 1933; BOURNE, 1945). The mitochondria of guinea pig liver tissue can be isolated (HOERR, 1943) and analysed. They are of lipidic nature (43.6 %) but contain at the same time two proteins of different I.E.P. They are free of lecithin and cephalin (BENSLEY and HOERR, 1934b). FAURÉ-FREMIET (1946) gives for the same object somewhat other figures: Protein 64.6 %, glycerides 28.8 %, lecithin and cephalin 4.2 %, cholesterol 2.25 %. At any rate there is no nucleic acid present. According to MONNÉ (1942b) the mitochondria may be strongly hydrophilic.

The rodlet shape of the so-called chondrioconts and the double refraction of the filamentous mitochondria from the intestinal cells of *Ascaris megalocephala* (GIROUD, 1928) indicate an inner structure which is at least of the kind of a mesophase. CLAUDE and FULHAM (1944) have published electron microgramms of fixed chondriosomes showing a lipid cortex and a watery less dense central zone.

Originally BENSLEY (1937) thought that the chondriosomes might be merely coacervates. But in recent publications they are considered as independent bodies with special physiological functions (CLAUDE, 1944), as certain enzymes have been found to be fixed on the chondriosomes.

It is difficult to decide, whether the analysed mitochondria have always been absolutely separated from every other cell constituents. In the liver cells of the guinea pig there occur besides the microscopic chondriosomes submicroscopic particles, which can be separated from the plasm and have an essentially different composition. In addition to 80–90 % of water they contain protein, nucleoprotein, flavoprotein, triglycerides, lecithin, sterine and vitamin A (BENSLEY, 1943); for this reason they are believed to be of particular importance as carrier for ferments and vitamins in the metabolic process.

Chromidia. The framework of the cytoplasm of sea-urchin and *Tubifex* eggs consists of coarse plasm fibrils, with microscopic dimensions (MONNÉ, 1946b; LEHMANN, 1947). They associate to double refracting, optically negative bundles. The negative sign is caused by lipids, which can be removed, whereupon the double refraction disappears. The fibrils carry small bodies of ribonucleic acid; the diameter of which is almost submicroscopic. These bodies have been termed as *chromidia* by

HERTWIG. The plasm fibrils appear to be segmented by them and display in this way a similar microscopical structure as the chromonemata (s. p. 144).

It is probable that the plasm fibrils multiply by longitudinal splitting. This would mean an autocatalytic self-multiplication similar to that known of virus nucleoprotein and chromosomes. Therefore LEHMANN (1947) considers the chromidia carrying plasm fibrils as *biosomes*, i.e., as structural and functional elementary units.

By definition biosomes would be plasmatic elementary units of complicated chemical composition (proteins, with additional compounds as nucleic acids or lipids), special structure, capable of self-multiplication and endowed with specific functional tasks. LEHMANN (1947) thinks that chromonemata, plasm fibrils with chromidia, and mitochondria are such biosomes. The given definition covers also the properties of the plastids in plant cells, although they are of a bigger size.

If the biosomes are compared with the virus nucleoproteins, it must be emphasized, that the virus does not possess a self-governed metabolism (W. FREI, 1943), but that its "life" is dependent on living cells, wherein it is a parasite. This is not true for the biosomes, so that a self-reliant metabolism should be attributed to them.

To my mind there is a difference of individual particles as mitochondria, plastids and chromonemata on one side and the plasm fibrils on the other side. The first maintain their individuality throughout the life of a cell, whilst it is difficult to prove this for the plasm fibrils. In the plasm sol they will be individualized and almost independent from each other, but in the plasm gel probably not. LEHMANN (1946) stresses the fact, that the morphogenetic pattern of an egg is due to especially structurated plasmatic districts which are highly resistent to deformation by high speed centrifuging. Were the plasm fibrils only losely interwoven those structures could hardly be so stable. It is probable that in this case fibrils are amalgamated by some kind of junctions.

WIESNER thought, that even the membrane of plant cells was built of individual particles, the *dermatosomes*. By indirect methods (Fig. 56) and by electron microgramms (Fig. 87), it can be shown, that no such unities exist. Between the micellar strands of cellulose (50 Å diameter) or even cellulose molecules and the wall of the total cell, there is no definite entity, as fibrils and microfibrils are only arbitrary fractions of the coherent structure of the whole cell wall.

Biosomes are morphologically definite units in between the range of macromolecules and the size of cells. It will be interesting to find out, whether in the plasm gel such units really do exist.

f. *Molecular Morphology of the Cytoplasm*

In this monograph the explanations of molecular morphology have been kept very vague and general on purpose. We have mentioned polypeptide chains and their junctions, lipophilic and hydrophilic groups, acid and basic side groups. This suffices to understand the general properties of the cytoplasm, but its specific achievements cannot be approached in this manner and require a knowledge of the exact configuration of the molecular framework. For such an approach, however, only *one* important starting point is available: the asymmetry of the cytoplasm. Of the stereoisomeric amino acids only the laevo forms occur in the cytoplasm (GAUSE, 1936) and accordingly, also the syntheses and the degradations which are carried out in the cytoplasm are strictly specific: of the possible isomers, only a special one is formed. Whereas artificial syntheses of an organic compound with asymmetric

carbon atoms lead to an optically inactive racemate, only the dextro or the laevo form of the same substance are formed in the cytoplasm.

This discovery of PASTEUR's is of far-reaching importance for morphology, for it shows how new configurations result from those already present: in the cytoplasm each structural creation requires an adequate creator. This is the principal reason why the cytoplasm cannot have the nature of a shapeless liquid, but must possess a framework of well-defined molecular structure.

Apoenzyme — Coenzyme with prosthetic group (Nicotinic acid amide) — Prosthetic group hydrogenised

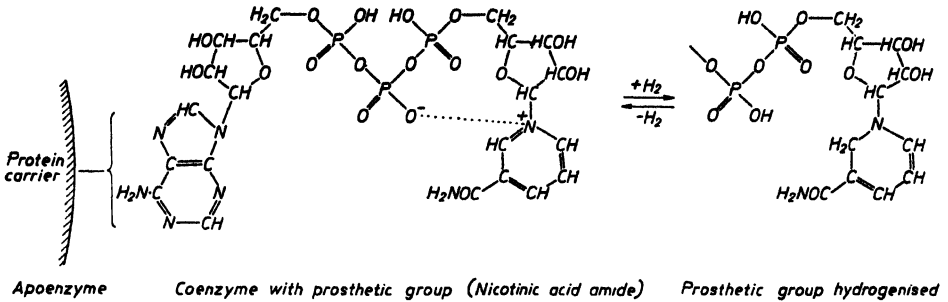



Fig. 110. Structural formula of dehydrogenase as an example of a co-ferment.

In addition to the asymmetry of the amino acids, which in the scheme of Fig. 88 is evident from the mutual positions of the H and R groups, numerous other structural particulars must exist in the cytoplasm framework. At present, however, we dispose of no means, such as optical activity, to discern them. Most certainly, however, all specific physiological reactions are caused by them. It has already been pointed out that *ferments* must carry such groups of a specific structure. In Fig. 110 an example is shown, concerning the ferment *dehydrogenase*, which acts as catalytic carrier of hydrogen in respiratory and fermentative processes. The active group of the molecule consists of a nucleotid (adenine, ribose and phosphoric acid, see p. 137), which is linked with a second nucleotid-like compound (nicotinic acid amide, ribose, phosphoric acid) by a molecule of phosphoric acid (KARRER, 1941, 1944). The nicotinic acid amide is capable of taking up hydrogen, and is therefore designated as active group or prosthetic group. It can, however, develop its activity only in unison with the total molecule and only on the condition that the latter is connected with a colloid protein carrier. The carrier is designated as *apo-ferment* and the molecule with the prosthetic group as *co-ferment* (compare for instance BERSIN, 1939). The two parts of the ferment can be chemically separated and recombined. In contrast to some co-ferments, the constitution of the apo-ferments is still completely unknown. In the so-called lyo-enzymes, which leave the cells and are active in solution, the apo-enzyme is a corpuscular protein particle of colloid dimensions. It must, however, be supposed that in the endo-enzymes, which are active only in the cells and can be isolated only by autolysis, i.e., by breaking down the colloid framework of the cytoplasm, the apo-ferment is anchored onto the molecular framework of the living protoplast. For this reason no discreet colloid particle with the function of apoferment does exist; the ferment carrier forms part of the coherent submicroscopic cytoplasmic framework.

Vitamins often contain specific structural units which are necessary for the formation of co-ferments, but cannot be formed by the heterotrophic organisms, since the latter apparently lack the formative principle indispensable to the synthesis

concerned. And also in the activity of *hormones* such molecular morphological particulars might play a part.

In this context the group of auxins amongst the phytohormones will be discussed briefly as a further example of compounds having a specific effect (WENT and THIMANN, 1937). The auxins admittedly are not very specific, since they initiate all kinds of different reactions of growth: elongation growth of merismatic cells,

I. Auxen triolic acid" Auxin A

II. Auxenolonic acid Auxin B

III. Indolyl-β-acetic acid Hetero-Auxin

IV. Inden-3-acetic acid

V. Indolyl-β-propionic acid

VI. Cumaryl-α-acetic acid

VII. Histidine

VIII. Ethylene

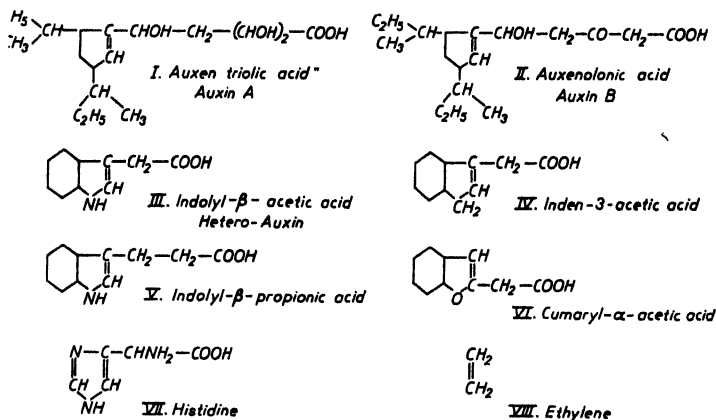



Fig. 111. Molecular structure of plant growth- and stimulant substances.

division growth of parenchyma and cambium cells, epinastic curvation of leaves, initiation of callus- and root-formation in cuttings, impeding of shooting out of axillary buds, etc. The experience, that chemically entirely different compounds stimulate the same or at least similar growth, created a still greater sensation than this diversity of positive or negative reactions caused by the growth substances applied. The nearly identical effects of auxin, an oxy-acid with a ring, a double bond and branched side chains, and of hetero-auxin, a heterocyclic nitrogen compound, are well-known. For this reason it has often been suggested that in the case of these stimulants the chemical process is of only secondary importance, or else that auxin and hetero-auxin cause different primary effects. According to GUTTENBERG (1942), for instance, hetero-auxin stimulates the formation of real auxin.

However, if one compares the 6 structural formulae of the compounds I–VI in Fig. 111 (THIMANN, 1936), all of which are stimulants of growth (although the compounds IV–VI are active to a considerably less extent), one observes that all have a morphological characteristic in common: they all contain a five-ring with at least one double bond. Six-rings (naphthyl derivatives) are also active (THIMANN and BONNER, 1938). Whether this ring is homo- or heterocyclic and what side chains or neighbouring rings are substituted in it, does not seem to be decisive. As a further characteristic which comes into consideration, it may be mentioned that all six substances are monobasic acids, in which however the COOH-groups must be separated from the ring by at least one C-atom (KOEPFLI, THIMANN and WENT, 1938). The morphological principle of the unsaturated five-ring seems to be particularly important. We do not know how this ring fits into the plasm structure, but it must possess a specific kind of stimulating activity, adapted to a certain configuration of the cytoplasm frame. It cannot be incidental, that histidine (Fig. 111, VII), the specific

stimulant to initiate plasm flow (FITTING, 1927, 1936), also shows the unsaturated five-ring, although admittedly with two double bonds.

Even the double bond alone is capable of initiating some of the reactions mentioned, for traces of ethylene (Fig. 111, VIII) cause typical epinastic curvation of leaves (which are even used as test reactions, DENNY, 1935), and give rise to the formation of adventive roots in the presence of a sufficient amount of auxin (MICHENER, 1935). For the initiation of cell elongation, however, to which end the cell wall must be plasticised by a dissolution of some of the junctions, the acid group too seems to be required. For the time being it is an unexplainable fact in molecular morphology, that the combination of double bond and acid group has to be realised by means of some five- or six-ring.

§ 2. NUCLEUS

a. *Molecular Structure of the Nucleus*

The isolation of sufficient quantities of substances from the cell nucleus for chemical purposes meets with great difficulties, and so far it has been possible to carry out a thorough chemical analysis only in special cases, in particular in the case of the sperm nuclei of fishes, where extremely interesting results have been obtained. The following account refers therefore primarily to fish sperm, but a generalisation to the chemistry of other nuclei on the strength of microchemical analogies is permissible, provided it is done in a cautious manner. The nuclear substances designated as nucleoproteins can be separated into two components: in proteins on the one hand and in phosphor containing nucleic acids on the other hand. Other compounds as, for instance, lipids (HIRSCHLER, 1942) are present in insignificant quantities. SCHMIEDEBERG (KIESEL, 1930) finds for the sperm heads of salmon:

nucleic acid	60.50 %	by weight
protamines	35.56 %	„ „
rest, with 0.12 % Fe . .	3.94 %	„ „

Protein components. Not without reason the presence of very complicated proteins was presumed in the nucleus, but contrary to expectation only relatively simple polypeptides, designated as protamines, were found in the fish sperm. They are characterized by the fact on hydrolysis they produce a striking number of basic amino acids, principally arginine, but also lysine, histidine and others. According to KOSSEL (1929) the proportion of the di-amino acids (compare Fig. 89) to the mono-amino acids: alanine, valine, leucine, etc. (abbreviated M) often amounts to 2:1. For example, in the case of the mono-protamines 2 arginine: 1 M; in the di-protamines 2 (arginine, histidine): 1 M; in the tri-protamines 2 (arginine, histidine, lysine): 1 M. Often the basic compounds preponderate even more. This preponderance of the di-amino acids results in polypeptide chains of strongly *basic character*. As a further characteristic KOSSEL mentions that the amino acids with 5 C members (ornithine, proline, valine) strongly come to the fore compared with those with 6 C atoms (for instance leucine), which are typical for other proteins. Still more important is the complete absence of cystine and amino dicarbo acids in the protamines.

For instance, the formula given in Fig. 112 is attributed to sturine from the sperm of the sturgeon, which represents a tri-protamine. KOSSEL (1905) tends to attribute special importance with regard to processes of procreation and formation

of organic substances to the alternating -C-N-C-N-order of the end groups of the side chains of arginine and histidine, which also occurs in the nitrogen containing bases of nucleic acids. For the time being, however, these facts can only be accepted as morphological statements, for the functioning of such systems is still unknown. In this context it seems noteworthy that the polypeptide main chain represents a -C-C-N-C-C-N-arrangement.

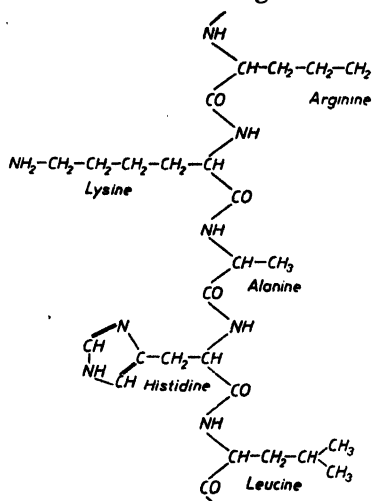



Fig. 112. Molecular structure of sturine.

The chains of the protamines obtained are not very long. For instance, salmine, with a molecular weight between 2000 and 2500, consists of only 15 to 18 amino acids (KIESEL, 1930), i.e., the polypeptide chain would contain only 45 to 54 groups. Undoubtedly, however, the polypeptide chains of the nucleoproteins will be much longer in the native state and will only fall apart in these short fragments as a result of the chemical treatment. The protamines seem to be strictly limited to fish sperm. In other nuclei, proteins, of less basic character have been found, the so-called histones, which have a higher molecular weight and are therefore less soluble. They contain a great variety of amino acids and form a transition to the typical proteins. Their I. E. P. lies in the alkaline region, up to a p_H of about 8.5 (PISCHINGER, 1937).

The ultraviolet absorption of proteins which results from the presence of cyclic amino acids (tyrosine, tryptophan, histidine) is small. The globulins, for instance, whose I. E. P. lies on the acid side show a weak absorption band at 2800 Å, whereas in the basic histones this band occurs at 2900 Å, which may be used as a means of identification (CASPERSSON, 1941). The histones appear to be concentrated in the nucleolus.

The nucleic acids also possess a pronounced chain structure. The chemical structure of the chain members, designated as nucleotids, is well-known. Hydrolysis leads to three components: one molecule of phosphoric acid, one molecule of sugar form the group of the pentoses and one heterocyclic basic ring from the pyrimidine or purine type. d-Ribose is the pentose of the majority of the nucleotids isolated, while all kinds of substituted pyrimidine rings (uracil, cytosine, thymine) or purine rings (guanine, adenine) can occur. Cytidylic acid, a nucleotid obtained from yeast, possesses for instance the structural formula shown in Fig. 113c.

Because of the purine and pyrimidine rings, the nucleic acids have a strong ultraviolet absorption, having its maximum at 2600 Å. This property has been very skilfully made use of in cytology by CASPERSSON (1936).

In the cells the nucleotids do not occur in the free state. A mutual esterification to polynucleotid has taken place, the latter representing the actual nucleic acids. The esterification takes place between an OH-group of the phosphoric acid and an alcoholic hydroxyl group of the ribose. It is possible that periodically, for instance after every fourth nucleotid, other kinds of bonds also occur. For example, in the

nucleic acid of yeast, four nucleotids (adenine, uracil, guanine and cytosine nucleotid) are combined into a tetra-basic acid. This nucleic acid, however, apparently does not occur in the nucleus but in the cytoplasm.

The nucleic acids from the nucleus differ from the nucleic acids of the plasm in that part of their nucleotids do not contain d-ribose but d-2-ribodesose. In this desoxypentose the OH-group at the 2nd C-atom of ribose has been replaced by H. It is

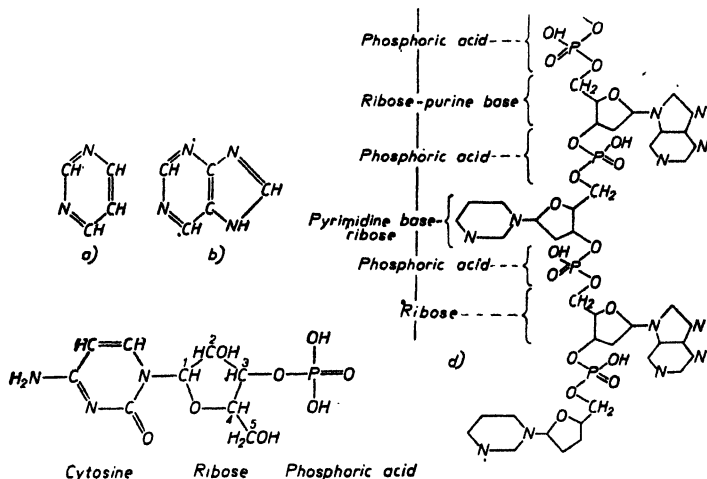



Fig. 113. Molecular structure of the nucleic acids. *a*) pyrimidine base; *b*) purine base (the rings are usually represented in the form of rectangles, but this might be incorrect from a morphological point of view); *c*) cytidylic acid = nucleotid cytosine-ribose-phosphoric acid (according to FISCHER 1942); *d*) nucleic acid = polynucleotid.

likely, that this small structural change causes the nucleic acids of the nucleus to be much more sensitive to hydrolysis. For, according to FEULGEN, after weak acid hydrolysis all nucleic acids of the nucleus show SCHIFF's aldehyde reaction with fuchsine in H_2SO_3. Obviously the hydrolysis of the nucleic acids of the nucleus liberates the aldehyde groups of ribodesose, whereas in the case of the nucleic acids of the cytoplasm the aldehyde groups remain masked. Accordingly this specific staining has been introduced in cytological microchemistry as FEULGEN's nucleal reaction to prove the existence of nucleic acids in the nuclei (FEULGEN and ROSSENBECK, 1924).

It has been possible to analyse macrochemically a number of nucleic acids showing positive nucleal reaction. *Thymonucleic acid* from the nuclei of the thymus gland consists of four nucleotids with the bases adenine, thymine, guanine and cytosine. A molecule of this relatively small size, however, will scarcely show colloid properties like the nucleic acids in the nucleus. It must therefore be supposed that the tetra-basic acid of Fig. 113d represents only a part of the native, high molecular, nucleic acids. Guanyl nucleic acid from the pancreas gland, the most complicated nucleic acid known at present, contains in addition to the tetra-basic thymonucleic acid a nucleotid with ribose as sugar and guanine as basic component. This shows that mixed polymerization products of nucleotids with ribose and ribodesose groups can occur in the nucleic acids of the nucleus. For further particulars we must refer to the literature concerned (for instance KIESEL, 1930; FISCHER, 1942).

Whereas formerly the nucleic acids were considered as tetra-nucleotids, it was suggested in the first edition of this monograph that they represent high polymer long chain molecules (Fig. 113d). SIGNER, CASPERSSON and HAMMARSTEN (1938) confirmed this by means of the birefringence of flow of Na-thymonucleate and simultaneously ASTBURY and BELL (1938) proved the existence of a chain lattice with a fibre period of 3.34 Å in artificial fibres of Na-nucleate. The degree of polymerisation is very high and only by taking special precautions it is possible to isolate them unimpaired from the thymus gland (KNAPP, 1946).

The main chain consists chiefly of P- and O-bridges; the phosphorus carries a free acid group, while the basic groups constitute short side chains. Compared with the nitrogen containing bases, the dissociation of the phosphoric acid preponderates to such an extent that the system represents a chain of polybasic acids. The isoelectric state lies below p_H 2 (PISCHINGER, 1937). The nucleotids of these main valency chains are no longer discernable as individual rod-shaped molecules or chain members in a morphological sense.

b. *Fine-Structure of the Nucleus*

Active nucleus. The nucleus possesses mostly a coarse framework. Its strands are usually of microscopic thickness, but as they are strongly hydrated and insensitive to dyes, they remain invisible in the living nucleus. Recently these framework fibrils have become of great importance, since they could be identified with the uncoiled chromosomes (compare p. 144). A sol-like liquid is found between the strands of the fibrils; it is designated as nuclear sap, karyolymph or enchylema. In other words, the structure is analogous to that of cytoplasm, where the frame of the plasm gel (kinoplasm, spongioplasm) is distinguished from the plasm sap (paraplasm, enchylema).

MARTENS (1927/29) and PISCHINGER (1937) have elucidated the connection between the invisible fine-structure of living nuclei and the visible structure of fixed nuclei. On fixing, the fibrils of the nucleus are dehydrated and become accessible to staining. They usually clot together as a result of the adhesive action of the coagulated proteins of the enchylema.

The framework structure in the nucleus has received a much more appropriate name than in the cytoplasm, where the wrong concept foam or honeycomb structure is often used. For it is designated as *reticulum*[1], which clearly expresses that both framework substance and karyolymph are continuous structural components. No vesicles or alveoles, in short no corpuscularly dispersed structural elements, are present. We may have to deal with an unusual coarsening and also with deformations brought about by shrinkage of the original framework, but these are no products of coagulation like the sediment of a protein precipitation carried out in vitro. The fact that the most different means of fixing reveal the same or similar nuclear structure already makes the pre-existence of the framework likely.

The living reticular framework is not rigid, but to a certain degree is plastic. By means of centrifugal forces NĚMEC (1929) has displaced the nucleolus in the nucleus, or even removed it altogether, in which case the reticulum was deformed. According to several authors the nucleus possesses the nature of a liquid (for instance SCHAEDE, 1927) or even no structure at all. This is derived from deformability and

[1] Most often the designation reticulum is applied only to the aggregated framework in fixed nuclei; in my opinion this term would even better characterize the loose framework consisting of chromonema fibrils in the living nucleus.

optical homogeneity. The spherical form, the capacity to form drops and the fact that living nuclei are often optically empty are put forward as further arguments. For this reason it has to be repeated that the behaviour of a colloid, whether elastic like a gel or more liquid like a sol, does not in itself allow of a decision as to whether or not it possesses a submicroscopic structure. To this end measurements of structural viscosity would be necessary, and the highly viscous nuclear substance certainly possesses this property to a large extent.

It is true that the anomalous viscosity could also be attributed to a mutual impediment of independent rod-shaped particles. Since the structural elements of the nuclei are represented by the uncoiled chromosomes, the question arises, whether they are embedded in the karyolymph as freely corpuscularly dispersed particles, or whether they form a definite structure. I am convinced that the latter is true, for in general the nucleolus remains in contact with the chromosome fibrils, on to which it has been condensed (HEITZ, 1931; GEITLER, 1940) and heterochromatin, if present, (compare p. 144) occupies a certain position in the nucleus and cannot be arbitrarily displaced.

The *karyolymph* (enchylema), on the contrary appears to be a sol. In *Allium* nuclei, for instance, LUYET and ERNST (1934a) succeeded in separating it from the framework substance of greater specific weight by centrifugal means.

According to LUYET and ERNST (1934b) the *nucleus membrane* which varies strongly in thickness is no self-consistent skin, but only a *phase boundary*. Other authors, however, mention a real envelope, the birefringence of which has frequently been found to differ from that of the nucleus itself. SCHMIDT (1939c) gives evidence of an optically negative spherite texture in the boundary layer of the nucleus (layer double refraction caused by protein chains running in a tangential direction). According to F. O. SCHMITT (1938) the sign of the spherite cross is reversed after imbibing with glycerol, urea or sugar solution; this would neutralize the layer double refraction, and the intrinsic birefringence of the lipids would become apparent. MONNÉ (1942c) believes the nucleus envelope to be a double membrane, consisting of a firm nuclear protein layer free of lipids and a very tender cytoplasmatic protein-lipid membrane.

The discoveries about the chemical structure of the nuclear components have to be brought into accord with the results of microscopic observations. As in the case of plasm, of the two principal components of the nucleus, the proteins come in the first place into consideration as structural elements of a framework. As a result of their unlimited capacity of polymerization and the manifold possibilities of bridge formation, the polypeptide chains are capable of forming structures ranging from the finest amicroscopic molecular framework up to the submicroscopic or even microscopic reticulum. Since the proteins isolated from the nucleus are strong bases, it could be expected that the polypeptide framework could easily be stained. The living nucleus however can hardly be stained without temporary or permanent damage (BECKER, 1936). For this reason it must be supposed that the basic groups, which occur as free groups in the isolated protamines and histones are screened off in the native state. If none the less one wishes to apply vital staining, these groups must be liberated by a slight hydrolysis. As in the case of the cytoplasm, it can be said that vital staining, brought about by the formation of coloured salts with basic or acid dyestuffs always means hydrolytic intervention; e.g., vital nucleus staining in a dilute solution of erythrosin acidulated with acetic acid.

It is reasonable to assume that the basic protein groups are screened off by nucleic

acids. Apparently, however, the active nucleus contains this component rather sparingly, so that other anionic substances must also take part in masking the basic groups of the polypeptide framework. Active nuclei are less intensively stained by the nucleal reaction than those which are subject to division. A more convincing proof is, however, brought forward by CASPERSSON's experiments (1936), which are based on the absorption of ultraviolet light by nucleic acids. By means of microphotometric measurements he shows that the concentration of nucleic acids in the nucleus strongly increases in the preliminary stage of cell division, to decrease again during the telophase.

It is possible that it is partly due to changes in nucleic acid content that fixed nuclei are sometimes more easily stained with acid dyestuffs (erythrophily), at other times with basic ones (cyanophily), as has been summarized by TISCHLER (1921/22). CASPERSSON however has not been able to establish a relation between nucleic acid content and basic or acid nuclear reaction with respect to dyestuffs in the nuclei of the gland of the oesophagus of *Helix pomatia*. This must probably be explained by the fact that not only the number of acid or basic groups in the nuclear framework, but also the p_H of the interstitial liquid is determinative for the anionic or kationic behaviour of the latter (KELLER, 1932; BECKER, 1936). On the other hand, the nucleic acid content probably determines the I. E. P., so that at a constant p_H value the adsorptive power of a nucleus toward basic or acid dyestuffs may vary.

CASPERSSON's photometric determination of nucleic acid seems to prove that the increasing chromophily of the nucleus is related with the accumulation of nucleic acids in the preliminary stages of cell division. It seems to me that the older cytologists who in the nuclear substance distinguished a component like plastin, linine or achromatin (difficult to stain and hardly digestible) from the easily stained „chromatin", already recognised the existence of the two fundamental principles in the nuclear structure: on the one hand a high polymeric, relatively resistant protein framework and on the other hand a compound, very sensitive to basic staining, the nucleic acid, which predominates during nuclear division but falls into the background in the active nucleus. The well-known staining of fixed nuclei with basic dyestuffs indicates the presence of liberated acid groups and the nucleal reaction points to aldehyde groups. Undoubtedly therefore, the chromatic substance consists mainly of nucleic acids. In spite of this it is not possible to designate these chemically well-defined compounds as chromatin. For, in cytology, the term chromatin has become a morphological concept for regions, showing identical behaviour with respect to staining (HEITZ, 1935). Those regions of the active nucleus or parts of chromosomes which after division do not lose their high nucleic acid content, are designated as heterochromatic (positively heteropycnotic, WHITE, 1945). In other words, ***heterochromatin*** comprises those thymonucleic acids which remain passive during the changing phases of mitosis, whereas chromatin or euchromatin consists of thymonucleic acids which in the process of nuclear division first increase and afterwards decrease again. It has been found that the heterochromatic regions (chromocentres) of a nucleus represent chromosome parts which locally have preserved their spiral structure (STRAUB, 1943).

To sum up it can be said that the reticulum consists of a protein framework in which varying quantities of nucleic acids are embedded. CASPERSSON (1936) has proved this by digestion experiments with giant chromosomes. The protein base can be destructed by activated trypsine solutions, whereas a previous treatment with lanthane acetate preserves the regions containing nucleic acid in the form of lanthane thymonucleate. Since the chromosomes originate from the reticulum, this result may also be regarded as an indirect proof of the structure of the nuclear framework.

The two components, protein and nucleic acid chains, do not only show opposite chemical behaviour in that the ones are positive (kationic) and the others negative (anionic). Incidentally their optical reactions are also opposite. In the natural state all polypeptide chains investigated so far are *optically positive*, whereas, according to the interesting model experiments of SCHMIDT (1937a) and in experiments of flow (SIGNER, CASPERSSON and HAMMARSTEN, 1938, WISSLER, 1940), artificially prepared fibres of the sodium salt of α-thymonucleic acid are *optically negative*. For this reason long-shaped nuclei with a high nucleic acid content, as for instance certain sperm nuclei (Fig. 116a) are optically negative. The negative reaction in polarised light is, however, limited to the chromatic part of the sperm head. Often the achromatic ends and connecting part are optically positive. The attempts to explain this positive reaction as rodlet birefringence, i.e., as positive textural double refraction, is not conclusive, as long as one does not obtain indisputable WIENER curves. And besides, since it has been proved (SCHMIDT, 1937a, p. 87) that these regions show positive intrinsic double refraction, it seems to me that the anisotropy must be attributed to the submicrospopic protein framework. I do not doubt that it exists also in the chromatic part of the sperm head, where it is more than compensated, however, by the strongly negative nucleic acid. If it were possible to eliminate the nucleic acid components completely without disturbing the structure and to dehydrate the protein to a sufficient extent, both the positive rodlet birefringence and the positive intrinsic double refraction of the protein framework would become apparent. The positive double refraction of achromatic oblong nuclei, such as the fibrous spindle-shaped nucleus of *Aloe* described by KÜSTER (1934b) must doubtless be attributed to the orientated protein framework. It seems to me that the chemical dualism in the nuclear structure is clearly demonstrated by its optical anisotropy, since the character of the double refraction is determined alternatively by the optically positive protein frame or the optically negative nucleic acid inclusions.

Our knowledge of the submicroscopic nature of the *nuclear sap* is less advanced. Decidedly it also contains proteins which probably are of somewhat lower molecular weight than the polypeptide chains in the nuclear frame and occur therefore as dispersed particles in sol- or gel-solutions. It has been proved that the nucleoli have their origin in the accumulation of these proteins (in particular histones, CASPERSSON, 1940a; SERRA and QUEIROZ-LOPES, 1944), which in that case could be regarded as reserve substances. As will be shown on page 216, the reserve proteins differ from the framework proteins by a lower degree of polymerisation and the globular form of their molecules. They may be arranged into molecular crystal lattices capable of swelling. It is significant that the nucleoli in the nucleus can be substituted by protein crystalloids (KÜSTER, 1935a, page 135) as is the case with representatives of the Scrophulariaceae (GICKLHORN, 1932b) and Lentibulariaceae. Sometimes nucleoli and crystalloids occur simultaneously in the same nucleus (ZIMMERMANN, 1896).

The principal difference between the proteins of the reticulum and those of the *nucleoli* is the greater solubility of the latter. In contrast to the very resistant nuclear frame they are readily solved by pepsin in hydrochloric acid. In spite of its high histone content the nucleolus apparently possesses weak anionic properties, for it vigorously collects most basic dyestuffs and as a rule shows a greater resistance against swelling in dilute alkaline solutions than the reticulum (TISCHLER, 1921/22, p. 45–51). On the other hand it stores eosin. Its behaviour towards dyestuffs depends strongly on the process of fixing and the method of staining (ROMEIS, 1943, p. 323).

It shows a specific affinity to the acid dye-stuff Methyl Green (Colour Index, 1st ed., nr. 684, in German Lichtgrün) which enables a differential staining in comparison with the red Feulgen reaction of chromatin (Semmens and Bhaduri, 1939). This double staining has become important for the problem of nucleoli formation in the telophase of cell division.

If the nucleoli represent reserve proteins, their formation is comparable to that of aleurone grains in the cytoplasm. In fact, it has been observed that the protein crystalloids which sometimes replace the nucleoli grow in small vacuoles of the nucleus. The place where the nucleoli, appear is predetermined for they condense in contact with special chromosomes provided with secondary constrictions (Heitz, 1935; Håkansson and Levan, 1942). At first they behave like real vacuoles, for in the presence of several chromosomes condensing nucleoli ,the several nucleoli formed can subsequently unite to form bigger ones. At the present state of our knowledge the nucleolus formation must be considered as an accumulation of the karyolymph proteins at a definite spot, which takes place at the expense of energy, until a coacervate droplet rich in proteins is formed. Serra and Queiroz-lopes (1945) think that the nucleolus is capable to synthesize nucleoproteins.

Nuclear spindle. The microscopic structure of the spindle which becomes apparent in nuclear divisions has long remained an enigma. In fixed preparations spindle-shaped fibrillae are visible, some of which stretch from the one pole of the cell to its equator, while others, shorter ones, coalesce with the chromosomes at special points of attachment (the primary constrictions according to Heitz). In the living state [illegible]wever all this remains invisible; microscopically the spindles are homoge[illegible] ructureless and optically empty. Microsurgical interventions reveal a relatively rigid double cone with distinct cleavability but without a visible structure (Belar, 1929). Accordingly the spindle fibres have been considered as artefacts of the fixing process.

In this case it has been possible to elucidate the true state of affairs by means of submicroscopic methods of investigation. Schmidt (1937a) finds the spindles to be positively birefringent in living sea-urchin eggs. Thus the images visible in the fixed material prove to be real structures existing in vivo. Since the poles of the spindle behave like positive spherites whose rays can be followed nearly throughout the whole cell, they must consist of optically positive submicroscopic fibrillae arranged in circles. Undoubtedly the same fibrils stretch from each pole to the chromosomes. In the living state however they are not visible, either because they are not microscopically resolvable or because, as a result of their degree of swelling, they possess the same refractive index as the ground mass of the spindle cone. It is also possible that they anastomize submicroscopically and thus do not represent a drift of individual fringes but an elastic body. As a result of deswelling in the fixing process, the fibres are made visible, either by mere dehydration or by some kind of crystalline aggregation of neighbouring, anastomizing fibrils. In this way a more pronounced parallel alignment of birefringent elements is achieved, which causes a marked increase in optical anisotropy.

Since the fibrils are optically positive, it may be assumed that they consist of polypeptide main valency chains. In any case this hypothesis may be used as long as no contradictions arise. The spindles are formed primarily in the cytoplasm when the nucleus is still individualized. In some cases even cells devoid of nuclei are capable of forming spindles (E. B. Harvey, 1936). Since we supposed the molecular

frame of the cytoplasm to consist of polypeptide chains, we have no difficulty in deriving the spindle structure from the plasmatic frame structure. To this end the junctions must be loosened and the chains be made to run parallel. In fixed preparations this fibrillation of the cytoplasm can often be observed in the regions surrounding the poles. I fully realise that the transition will not take place according to this simple scheme, but must be connected with a conversion and reconstruction of protein chains. However, the principal condition is that the cytoplasm already contains the structural elements required, i.e., the polypeptide chains, either as structural material or as a model for the formation of new chains.

The spindle is not always formed outside the nucleus; in special cases it has its origin inside the nucleus, or it is observed that cytoplasmatic and nuclear fibrils together take part in the construction of the spindle. This once more indicates that the submicroscopic structures of cytoplasm and nucleus are alike. By submicroscopic changes fibrillar elements of similar morphological nature can originate from both of them. We may conclude that the nucleus chemically does not separate from the cytoplasm as a completely foreign substance.

The protein chain structure of the spindle fibres can be made use of in the so-called „strain theory" (Zugfasertheorie), according to which the chromosomes are drawn towards the pole by shortening fibres. In fact, stretched polypeptide chains have the property to contract considerably under certain circumstances (see p. 200). To these considerations one might object that in this case not only the fibrils connected with the chromosomes but also those seemingly stretching from pole to pole must be shortened. SCHMIDT (1939a) has shown however that the double refractio[illegible] of the spindle fibrils is extinguished at the equator. In other words, the fibr[illegible]s ru[illegible]m pole to pole appear to be interrupted. The intermediate body formed at the equator (phragmoplast) is isotropic. If the chromosomes are drawn apart from each other, the double refraction of the fibrils decreases, as is to be expected in the contraction of protein fibres.

Chromosomes. The chromosomes differentiate from the nuclear framework wherein they are preformed. During the prophase of cell division they disentangle, shorten and become independent. The membrane of the nucleus is not merely a phase boundary like the surface of contact between vacuole and cytoplasm, but rather a solidified peripheric part of the nuclear sap. Accordingly, after the destruction of this boundary, the karyolymph readily mixes with the cytoplasm. Since it disappears after the formation of the chromosomes, it is obvious that its polypeptide material does not take part in the construction of the latter; either it is changed into soluble protein molecules or used in the fibrillar frame of the spindle

Often the chromosomes possess two arms. The connecting part between these arms is somewhat constricted (primary constriction) and cannot be stained by FEULGEN's reagent; it is anucleal. The constriction serves as point of contact for the spindle fibre. This region often possesses a clearly visible boundary and is then designated as centromere. In addition to the primary constriction one often finds so-called secondary constrictions, where the nucleoli condense during the telophase. Fig. 114a further shows some heterochromatic parts (end of left chromosome arm and satellite).

On the basisof the chromonema theory a clarifying hypothesis about the submicroscopic structure of the chromosomes can be developed, supported for instance by GEITLER (1934, 1938, 1940) and HEITZ (1935) in their elaborately documented

summarizing studies on the structure of chomosomes. Each chromosome contains one or two, according to more recent investigators (NEBEL and RUTTLE, 1937; NEBEL, 1939, 1941) even four spirally wound fibrils, designated as *chromonemata* (Fig. 114a). In the latter case they are identical with the *chromatid fibrils* (Fig. 114b), well-known from the prophase of reduction division. It is only in this state that the chromonema spiral is uncoiled and therefore surveyable in its entire length which

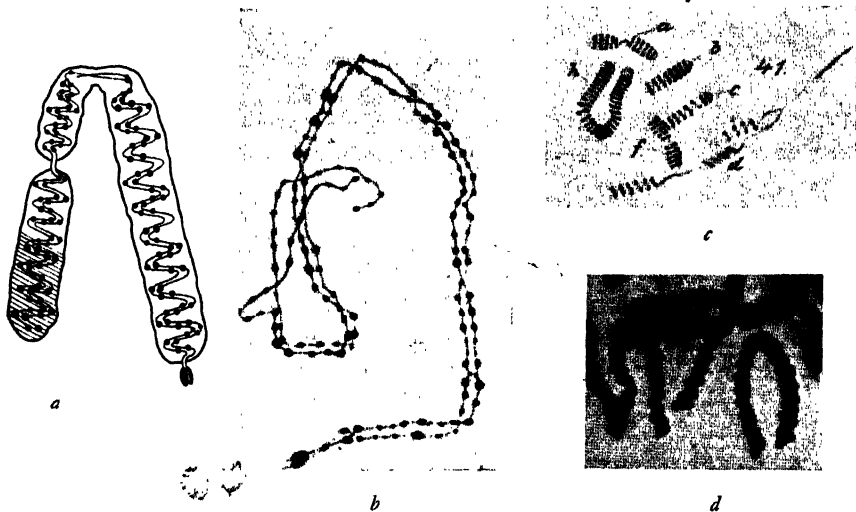



Fig. 114. Microscopic chromosome structure (according to HEITZ 1935). *a*) Idealised chromosome with helicoid chromonema fibrils; heterochromatic region hatched; in the upper part: a primary (kinetic) construction; in the lower part at the right: secondary constrictions with satellite (corrected after GEITLER's criticism 1938, p. 98); *b*) Chromatid pair of *Trillium erectum*; *c*) helicoid structure of the chromosomes of *Tradescantia virginica*; *d*) helicoid structure of the chromosomes of *Trillium erectum*.

many times surpasses the length of the chromosome. It consists of an anuclear fibril which at regular intervals is covered with knots showing the nucleal reaction, and which are designated as *chromomeres*. In the chromosomes these particulars can scarcely be observed, since the coils of the chromonema fibrils are lying on top of each other (Fig. 114c) and besides are embedded in a ground mass (matrix) which shows strong nucleal staining. It must be mentioned, that a spiral structure has been observed only in large chromosomes. According to Japanese and American cytologists (STRAUB, 1938; HUSKINS, 1941, 1942) the visible helix, the chromonema, sometimes possesses a spiral structure of its own, in which case the chromosome would possess the structure of a *double winding helix* with a primary and a secondary spiral (large and small spiralling). With the aid of the phase contrast microscope RUCH (1945) has shown that in the case of the frequently investigated chromosomes of *Tradescantia* the chromomeres occurring in pairs on the spirally wound chromonema fibrils delusively suggest the existence of the fine spiralling, if the microscopic resolving power is inadequate. The question as to how the helical chromonemata are separated from each other during mitosis without decoiling is a problem in itself (MATTHEY, 1941).

The chromonema theory has gained general importance by the discovery of the giant double chromosomes of the nuclei from the salivary glands of the Diptera. In

these marvellous cytological objects homologous chromosomes are united into astonishingly broad and remarkebly long ribbons. By means of dye-stuffs or the nucleal method numerous crosslines are made visible in this tape (Fig. 115). These gigantic structures may be regarded as bundles of numerous chromonemata, formed by endomitosis (HEITZ, 1935). They are united into strings of considerable dimensions; the chromomeres form round transversal discs.

The non-stainable, anucleal regions of the chromonemata bundles represent the protein components of the nuclear frame. From their fibrillar structure it may be concluded that the submicroscopic system of polypeptide chains is not restricted to the colourless segments, but runs invisibly through all the chromomeres in every chromosome fibre. In the chromomeres the nucleic acid components are localized, thus masking the protein ground mass. Their localization is demonstrated by the nucleal reaction and the ultraviolet absorption. Moreover the ultraviolet microscope with its higher resolving power allows of proving the existence of the protein ground mass of the chromomeres. With the aid of the digestion experiments mentioned on p. 140 in which the nucleic acid was protected from digestion by lanthane thymonucleate, CASPERSSON (1936) finds that the chromomeres are resolved into extremely thin discs. Ultraviolet photography reveals a fine-structure of lamellae with a thickness of only 0.1 μ. Since at this order of magnitude the limit of the resolving power in ultraviolet light is reached, the question as to whether these very thin chromomere discs possess a still finer submicroscopical structure and thus are subdivided remains unsettled. Personally I do not doubt that they are.

Fig. 115. Giant chromosomes of the nuclei from the salivary gland of a *Drosophila* hybrid with a chromosome structure characteristic of the two parental species (according to PÄTAU 1935): mel from *Dr. melanogaster*, sim from *Dr. simulans*; in *a* a structural difference.

Conversely, milt nuclease digests the nucleic acids of the chromomeres (MAZIA and JAEGER, 1939) without disturbing the ground structure of the chromosomes of the salivary glands. The stainability according to FEULGEN disappears; on the other hand the ninhydrine test turns out positive over the entire length of the chromosome. So the chromonema does not consist of alternating protein and nucleic acid links, but represents a continuous protein fibre in which at regular intervals nucleic acid knots are intercalated. The nucleic acids form saltlike compounds with the protein ground mass, the nucleoproteins, whose occurrence is therefore limited to the chromomeres (Fig. 116b–d).

Fibrillar hypothesis. From a morphological point of view WRINCH (1936) believes the molecular structure of the chromonema to be as follows: the polypeptide chains form a system of parallel fibrils like the warp of a weaving-loom and the nucleic acids represent the woof in this system of chains. Every four neighbouring polypeptide

chains are kept together by a molecule of the tetra-basic thymonucleic acid. Now the native nucleic acids certainly have a much higher molecular weight, i.e., the woof would not consist of short chains with four members but of longer chains. The heteropolar salt bonds between the acid groups of the nucleic acid chains and the basic groups of the polypeptide chains would have to be considered as the junctions of this network (Fig. 116b).

It is regrettable that this elucidating scheme is not supported by the optical properties. The artificial nucleic acid fibres obtained by SCHMIDT (1937a) are optically negative; and since in the spinning of fibres the molecular chains are arranged parrallel to the axis, the polynucleic acid chains themselves must also be *optically negative*. It follows from this that the molecules of the nucleic acid chains in the sperm nuclei (SCHMIDT, see Fig. 116a) run parallel to the morphological axis of the sperm head. But also the polypeptide frame of these nuclei must be orientated in the same direction. This means that the chain molecules of both nuclear components show parallel alignment.

a) b) c) d)

Fig. 116. Submicroscopic arrangement of nucleic acid (shaded); *a)* in the head of the spermatozoon of *Sepia* (according to SCHMIDT 1937a); *b-d* in the chromonema: *b)* transversal, according to WRINCH (1936); *c)* length-wise, according to SCHMIDT (1937c, 1939b); *d)* scattered orientations, according to FREY-WYSSLING (1943c, 1944a).

The chromomere discs of the chromosomes of the salivary glands are optically negative (SCHMIDT, 1937c, 1939b). For the submicroscopic fine-structure of the chromomeres SCHMIDT therefore takes into consideration a possible arrangement as given in Fig. 116c. The fact, ascertained by ASTBURY and BELL (1938), that the fibre period of 3.34 Å of the nucleic acids is about the same as that in the polypeptide chains (3.5 Å, see Table XXVI) seems to support this hypothesis (compare SERRA, 1943).

With the aid of the ultraviolet dichroism of the nucleic acid chains CASPERSSON (1940b) has checked the structure proposed in Fig. 116c. If the nucleic acid molecules in the protein fibres showed complete orientation, chromomeres, like artificial *thymonucleic acid fibres, would display a very pronounced dichroism in polarised* ultraviolet light. Compared with these fibres, however, the chromomeres of the chromosomes of the salivary glands show only an extremely small dichroitic effect. *CASPERSSON therefore draws the conclusion that the nucleic acid chains are intercalated practically without orientation. Also the double refraction of the chromomeres, as derived from the birefringence of flow of sodium nucleate sols* (SIGNER, CASPERSSON and HAMMARSTEN, 1938) proves to be very small. Meanwhile, premising that nucleic acids are straight chains, the negative sign of the chromomere birefringence indicates that the chains have a certain preference orientation. With the aid of the formula mentioned on p. 63 the scattering in the orientation of the chain molecules can be calculated (FREY-WYSSLING, 1943c), and the scattering angle found in this way is 86°.5, i.e., nearly a right angle. This means that the scattering is almost complete, thus furnishing an important argument against the supposition that the

nucleic acid molecules are parallel to the chromonema axis. A similar result is obtained if the intrinsic double refraction of −0.050 found by SCHMIDT (1928) for the chromatin of the *Sepia* sperm, or even only a fraction of this value, is compared with the birefringence of the chromomeres.

In spite of the insufficient orientation of the nucleic acids, CASPERSSON assumes the protein chain structure to be continuous. Orientated polypeptide chains should cause the anucleal chromosome segments to appear positively birefringent. This effect, however, can only be observed in stretched chromosomes (F. O. SCHMITT, 1938; PFEIFFER, 1941a). Optics therefore do not allow of gaining sufficiently reliable data to assume orientation of the protein chains. All the same the anisotropy of swelling and the cleavability of the chromosomes are in favour of an orientation along the long axis of the protein ground mass. In his microchemical experiments with chromosomes of salivary glands PAINTER (1941) is also impressed by their fibrillar character. It must be supposed that similarly to the isotropic segments of muscle fibres (compare p. 208) the isotropy of the protein chains result from coiling, folding, crossing, etc., combined with a corresponding hydration. Evidently the chromosome protein occupies a position intermediary between the fibrillar and the globular proteins (compare p. 219).

The explanation of the birefringence of other chromosomes is much impeded by our insufficient knowledge of the submicroscopic orientation in the chromonemata bundles of the salivary glands. According to BECKER and KOZBIAL (1937) the optical character of the chromosomes of the root tips of *Allium* and *Vicia* depends on the process of fixing: if treated directly with alcohol they appear to be negative; after a previous treatment with acetic acid vapour (swelling!) they are positive. On the assumption of a nearly complete scattering of the nucleic acid chains in the isotropic living chromosome these effects might be explained as tendencies toward orientation as a result of the shrinkage or swelling in the fixing process. It seems to me that considerations of this kind open more prospects than explanations formerly attempted with the aid of the spiral structure (NAKAMURA, 1937). KUWADA and NAKAMURA (1934) explain the positive double refraction of the chromosomes of *Tradescantia* by a single spiral of negative chromonemata; whilst, to their mind, optically negative chromosomes should be caused by a double wounded spiral.

c. *Submicroscopic Morphology of Hereditary Processes*

Genes. The fibrillar character of the chromatids meets two important morphological requirements of genetics: 1. the substrate is *easily cleavable in the direction of the long axis*, which is not only necessary for the splitting of chromosomes but in the first place also for the phenomena taking place between synapsis and diakinesis in heterotypic division; 2. the long chromonemata offer an opportunity for the *linear arrangement* and the possibility of *exchange of the genes.*

MORGAN's school has calculated that the number of genes known in the *Drosophila* chromosomes is so large that for reasons of space each gene must be bound to relatively small molecules of about the same order of magnitude as found in the reserve proteins (compare Fig. 155) investigated in SVEDBERG's ultracentrifuge. However, it would hardly be comprehensible how such freely moving particles are able to intervene decisively in the processes of development. To this end their carriers must have fixed mutual positions and it is best to imagine that they are fixed onto submicroscopic protein fibrils. In this way we comply with the

requirement of linear arrangement in a manner which could hardly be improved.

In spite of its great probability, however, an irrefutable proof of the existence, of the submicroscopic fibrillar structure has not yet been given. As has been shown the quantitative evaluation of the optical results suggests that the basic protein is in an isotropic state rather than in a pronounced fibrillar state (possessing the characteristics of a chain lattice); and thus far the electron microscope has failed, because even the pachytene and diplotene chromosomes yield only compact black shadows (ELVERS, 1943a) showing less particulars than a good optical image. It is of so much the greater value that the experimental investigation of mutation or ray genetics (ZIMMER and TIMOFEEFF-RESSOVSKY, 1942) opens new perspectives.

Target theory. Artificial mutations are induced by ionising rays (UV-rays, X-rays, γ-rays). The dose of rays is measured by the X-ray unit r, which is defined as that amount of rays which will bring about enough conductivity under prescribed conditions in a chamber of 1 cm^3 of air to permit a charge of one electrostatic unit to be measured at saturation current. It is now established that the mutation rate induced artificially by radiation is proportional to the dose of rays brought to bear (TIMOFEEFF-RESSOVSKY, 1940). The effect is independent of the wavelength and the dose (intensity $\times$ time) can be irradiated all at once, concentrated or diluted, or else fractionated at optional intervals. There appears, therefore, to be no recovery. For instance, whereas the sex-linked mutations in the X chromosome of *Drosophila* have a natural mutation rate of approximately 0.2 %, the irradiation of 2500 r liberates a rate of 7 % and 5000 r liberates 14 %. If the mutation rates are plotted as a function of the dose, a straight line results which intersects the zero point; thus there is no threshold value and any small dose will take effect.

Ionisation consists in the formation of ions from neutral molecules by the action of irradiated energy. The molecules in question are, as it were, struck by the energy quanta of the radiation and are thereby modified. That is why the occurrence of a single ionisation is called a *hit*. The relation between mutation rate and dose of rays indicates that a mutation is the result of such a hit. It can also be demonstrated (TIMOFEEFF-RESSOVSKY, 1940) that the interdependence of dose and rate would not produce a straight line if several hits were needed to bring about one mutation. The conclusion to be drawn from the biophysical analysis of chromosome irradiation is, therefore, that the artificial mutation of genes is the elementary result of a single hit. There are, it is true, other possible physical explanations, besides the target theory, which may account for the effects observed (MINDER and LIECHTI, 1945).

The approximate number of atoms in a cubic centimetre of organic substance being known, and also the number of single ionisations which a r unit is able to evoke, it is possible to calculate how many atoms are needed for one of them to be hit to produce the mutation in question, this by means of the experimentally ascertained mutation constant, which indicates the degree of probability to incite a mutation by a given dose of radiation. The volume occupied by these atoms altogether is called the *target area*. It varies with different mutations of genes within the X chromosome of *Drosophila*; nevertheless an average can be calculated, according to which the susceptible volume amounts to $3.20 \cdot 10^{-20}$ cm^3., from which it follows that the radius of the target area (thought to be spherical) is .1.97 mμ (TIMOFEEFF-RESSOVSKY, 1940).

There is an alternative method by which the target area can be computed. If very strong ionising rays are used, of very great density, such as neutron rays, for example, more than one ionisation may take place in one target area, only one of which, however, effects mutation. The other ionisations are inoperative and the mutation rate must consequently be smaller than was to be expected from the irradiated dose of rays in r units. Indeed, in the case of the X chromosome of *Drosophila*, the mutation rate actually is 1.6 times smaller for neutron rays than for X-rays, with the same dose of rays. The

radius of the spherical target area can now be deduced from this factor together with the known density of ionisation for neutron rays; LEA (1940) finds 1.89 mμ. Seeing that this figure so nearly agrees with the value found by TIMOFEEFF-RESSOVSKY, it may be taken as fairly certain that the order of magnitude of the target area is roughly 4 mμ.

The target area is not to be identified with the gene, since it only gives us the size of a sensitive area within which something has to happen favouring the probability of a mutation. The gene may therefore be larger than the target area, viz., if not all parts of the former are capable of changing their molecular structure by ionisation. It may, alternatively, be smaller, if it does not attain the overall dimension of the statically calculated ionisation which, according to TIMOFEEFF-RESSOVSKY (1940), contains 100–2000 (with a mean of roughly 1000) atoms. The latter possibility is, however, discounted by estimations of the size of the gene made by specialists in genetics. True, it is often stated in the literature that the target area is of the same order of magnitude as that of the gene size found by other methods, but we shall show that this is not so.

One known estimation of the kind comes from MULLER (1935). Assuming that a single chromonema thread of the salivary gland chromosomes had the same volume as in the corresponding metaphase chromosomes of normal cells, the following calculation applies to the X chromosome of *Drosophila*. In the metaphase its volume is 1/8 μ^3, two-thirds of which fall to the share of the chromonema, the length of which in the salivary gland chromosome is 200 μ. When completely uncoiled, therefore, a single chromonema thread has the submicroscopic thickness (cf. METZ, 1941) of 0.02 μ. The thread is thinner still if it is assumed that the chromonema is regularly screwed-up in the metaphase chromosome, the diameter of which is $\frac{1}{4}$ μ. The length of 200 μ gives us 250 windings; consequently, with the chromosome being 2 μ in length, the chromonema could not be thicker than 0.004 μ.

In calculating the length of the chromonema section containing a gene, MULLER was guided by the following consideration: By examining the interchange of factors in cross-breeds, four genes were localised in a given chromomere of 0.5 μ width in the salivary gland chromosome and the existence of further genes was shown to be improbable. Thus the length covered by a gene on the chromonema thread would be about 0.125 μ. This is a dimension which lies on the borderline of microscopic resolving power. The chromonema sections which, according to MULLER, correspond approximately to one gene, are shown in diagram in Fig. 117d and, for comparison, the target area is indicated by a black circle. It is recognized that the thickness of the chromonema thread is of the same order of magnitude as the diameter of the target area, but never the estimated size of the gene, the volume of which exceeds that of the target area by two to three orders of magnitude! It can be shown that the sphere of action within a gene has a similar size as the target area.

Carrier hypothesis (FREY-WYSSLING, 1944b). If the volume of the gene is liable to be more than a thousand times larger than the target area, what, it must be asked, are the relations between these two quantities? It will be seen in Fig. 117d how the small, sensitive region is embedded in a large, non-mutating area. It is not known where the sensitive region lies and it may therefore, if desired, be thought of as placed anywhere. The picture is reminiscent of that of the *ferments*, where small active groups are similarly carried by a larger protein complex system (see Fig. 110). I therefore propose to discuss the picture of carrier and prosthetic region for the genes as well and to call this view the "*Carrier hypothesis*".

Since it is not the salivary gland chromonemata stretched to the utmost, with their hypothetical submicroscopic thickness, which operate in hereditary processes, but the considerably shorter meiotic chromosome threads (Leptonema, Zygonema), the size of the gene should be derived from the conditions produced by reduction division. We learn from genetics that the regions inciting mutation are placed linearly in the conjugating chromatids; consequently the target areas must likewise be aligned lengthwise in the leptotenic chromosomes. As the X chromosome is supposed to contain about 1800 genes (TIMOFEEFF-RESSOVSKY, 1940), all sensitive regions of 4 mμ diameter should together produce a length of 7.2 μ. Bearing in mind that, according to MULLER (1935), the genetically active volume of the X chromosome is roughly $^1/_{12}$ μ^3, one finds for the thickness x of the extended leptotenic

chromosome prior to conjugation of the chromosomes, $\frac{1}{4}\ x^2 \cdot \pi \cdot 7.2 = {}^1/_{12}$, from which $x = 0.12\ \mu$ is derived for the thickness of the so-called leptonema. This value may be of the right order of magnitude, since the diameter of the leptonema is in the vicinity of microscopic resolving power.

If the genetically active chromomeres of the leptonema are now divided up into submicroscopic slices of the thickness of a target area, the region corresponding to a

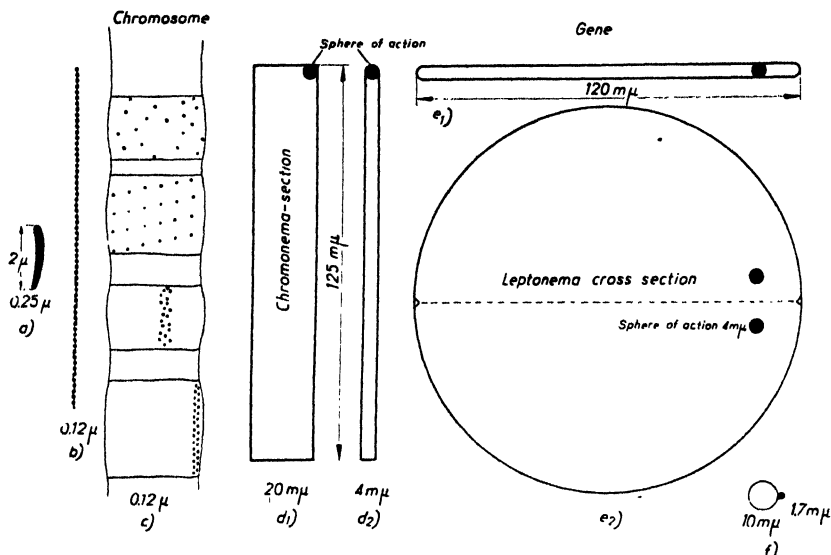



Fig. 117. Chromosome and genes. *a*) X-chromosome of the *Drosophila*; *b*) leptonema with linearly aligned chromomeres; *c*) leptonema, strongly magnified, with interchromomeres and target areas as points correspondingly magnified; *d*) size of genes calculated as prisms with quadratic cross-section according to MULLER (1935), each containing a target area in corresponding size, d_1 upper and d_2 lower limit of gene size; *e*) gene size according to the carrier hypothesis, at the same magnification as d; e_1 front view of the gene disc in comparison with a target area, e_2 ground-plan of the gene disc, indicating the twofold construction out of 2 chromatids with equivalent spheres of action; *f*) yellow respiratory ferment with apo- and coferment, both drawn on the same scale as d and e.

gene should be included. In this view and by this calculation, the gene should be a flat disc having an estimated diameter of 120 mμ and thickness of 4 mμ. We are completely ignorant as to where the target areas are in these discs: the arrangement may be any of an almost endless variety, as in Fig. 117c. The only certainty we have is that, given the linear alignment of the loci of the mutations, they must be juxtaposed in the axis of the leptonema. In Fig. 117e such a gene disc is represented to the same scale as the dimensions of the gene calculated by MULLER as prisms (1935). The position of the target area within the gene being unknown, it is shown as a globule placed arbitrarily anywhere in the disc. It is interesting to note that this size of the gene tallies well with that computed by MULLER (chromonema cross-section x length of gene), although found by totally different means (leptonema cross-section x diameter of target area).

Gene size acc. to MULLER (1935), Fig. 117d	(2000) – 50 000 $(m\mu)^3$
Gene size acc. to Carrier hypothesis, Fig. 117e	45 000 $(m\mu)^3$
Target area acc. to TIMOFEEFF-RESSOVSKY (1940)	32 $(m\mu)^3$

Scheme 117e is even more reminiscent than 117d of the structure assigned to the ferments. Fig. 117f depicts the yellow respiratory ferment to the same scale. Both the size of the colloidal carrier (mol. weight 70000) of this enzyme and its prosthetic group are known. Presuming that 1000 atoms occupy a volume of $3.2 \cdot 10^{-20}$ cm^3 (TIMOFEEFF-RESSOVSKY, 1940) and that, in accordance with the composition of sturine (Fig. 112, 27 C: 11 N: 5 O: 47 H), the average weight of the atoms of the amino acids is 5.6, the diameter of the apo-ferment (thought of as a sphere) is calculated to be about 10 mμ and that of the coferment with 81 atoms, approximately 1.7 mμ.

It will be clear from the following that a similar comparison applies to other ferments, at any rate so far as the prosthetic group is concerned:

Co-ferment of carboxylase (aneurinopyrophosphoric acid)	44 atoms
Co-ferment of the dehydrogenase II (nucleotid of nicotinic acid + phosphoric acid + nucleotid of adenine) hydrogenated	78 „
Co-ferment of the yellow respiratory ferment (lactoflavin-dinucleotid of adenosine)	81 „
Average target area of the genes	1000 „

The apoferments of these desmoenzymes are not freely moving colloidal particles; like the genes in the chromosome, they are embedded in the submicroscopic cytoplasmic structure. Only by autolysis can they be liberated under certain circumstances and made accessible to examination.

The comparison between gene and ferment may possibly not be merely an external one; one might at least try to probe further. It is scarcely to be wondered at that the gene and target area should be so much larger than the volume of the apo- and co-ferments, if it be remembered how much more complicated than single metabolic reactions are the processes of development controlled by the genes. Latterly it has become ever more evident that this control is exercised chemically. When a mutation takes place, these chemical processes proceed differently. It is therefore not wrong to assume that the target area acts like the prosthetic group of a ferment and that the controlled processes follow a different course owing to changes in this sensitive area. As this area contains approximately 1000 atoms, 50 amino acid residue (with on an average 20 atoms) are located in it, allowing protein chemistry to come into full play in its almost unlimited variety.

According to the hypothesis propounded here, the gene would, in the terminology of HAASE-BESSEL (1936), consist of a carrier (pheron) and chemically active regions (agon), some idea of the dimensions of which can be formed on the basis of the target theory. Since, however, every colloidal particle of the apo-ferments carries only one amicroscopic operative group, this conformity cannot likely be assigned to the genes. Probably there are several chemically active regions in the large disc of Fig. 117e. This would explain polyphaeny, i.e., that phenomenon whereby often more than a single phaenon is regulated from a locus of the chromosome. This view would also account for minor distinctions in activity of homologous active regions in different individuals and would explain polyallely. Finally, there must be some correlation between the carrier and the active region, in the same way as a coferment can only develop its activity in close conjunction with the apoferment; to some such reciprocal action must be attributed the different ways in which certain phaena are actualized. To be brief, the carrier model serves to make intelligible most of the knowledge acquired by research into heredity.

There being good grounds for assuming that the leptonema has a double structure, falling into two chromatids after conjugation, the carrier discs may be represented as two halves, each with an operative region of the same value (Fig. 117e). Of these two, only one need be struck by the rays for the origin of a mutated gamete, since the chromatids are separated from each other in the formation of tetracytes.

The advantage which the *carrier hypothesis* possesses, as compared to the *fibrillar hypothesis* developed in the chapter on chromosomes, is that it disregards the disputed question of molecular protein structure and nucleic acid intercalation; the elementary units may be conceived of either as fibrillar protein units coiled in any way, or as globular proteins. Then, the carrier hypotheses makes the gene and operative region of *submicroscopic* dimensions, whereas the fibrillar hypothesis allows the gene to be of *amicroscopic* size represented by side chain groups of polypeptide chains. This picture unwarrantably simplified, in the first edition, the actually exceedingly complicated facts. On the other hand, the fibrillar hypothesis has to its credit the plausibility it confers upon the shape, cleavability and self-duplication of the chromosomes. It will therefore be the aim of research to reconcile these two hypotheses to a concordant theory by endeavouring to fathom the submicroscopic morphology of those proteins which represent neither their extreme fibrillar, nor their purely globular form.

Function of the nucleic acids. The nucleic acids, which were at first thought to be the hereditary substance par excellence, are of relatively uniform chemical constitution and, in their molecular morphology, lack the diversity required by genetics. Moreover, CASPERSSON's measurements show that their appearance is transitory and that they afterwards largely disappear. For this reason KIESEL (1930, p. 185) stigmatises as downright paradoxical the fact that cytologists pay such conscientious attention to an unspecific material like the nucleic acids, yet ignore the proteins, with their specific structure, merely because they do not bind the basic dyes used for staining cell nuclei. POSTERNAK (1929) goes to the length of relegating the nucleic acids to the rank of degradation products of organic phosphorus compounds; but this view is invalidated by the morphological behaviour of the nucleic acids during karyokinesis and the interesting fact that many co-ferments consist of nucleotids (codehydrogenase II and others, see p. 133).

I have therefore suggested the following hypothesis respecting the function of the nucleic acids: The genes play no active part during karyokinesis, but are *passive* and in this state are distributed by some process among the daughter nuclei. Their operative groups must therefore be reactive in the active nucleus to fulfil their task, but they must be *screened off* during nuclear division[1]. This might be effected by a loose binding of nucleic acid groups. It was pointed out in the discussion of the phosphatids, that, in the respiratory combustion of carbohydrates, those hydroxyl groups of the sugar which are not subject to degradation are screened by phosphorylation and are thus temporarily protected. Similarly, the phosphoric groups of the nucleic acids might for the time screen the specific groups of the genes during mitosis. This would account for the localisation of the nucleic acids in certain places only, viz., where the genetically active groups are to be found in the fundamental protein substance. They thus give a true picture of the distribution of genes as proved by cytology. There is, then, nothing "paradoxical" about the attempts to establish the distribution of the nucleic acids in the chromonema down to the finest detail, since

[1] KOLTZOFF (1938) also regards the nucleic acids as "protectors" in this sense.

these are the indicators, as it were, of the specific groups through which the genes operate.

The assumption that the nucleic acids accumulate only in those parts which contain genes, and protect their active groups, integrates the conflicting views championed by the theorists of heredity, one being founded on the structural chemical specificity of the proteins, whereas the other side upholds the micromorphological specificity of nucleic acid distribution.

The fate of the nucleic acids in the cycle of nuclear division favours the above hypothesis. When the nucleus undergoes mitoses, nucleic acids are built up (increasing chromophilic tendency, nucleal reaction and ultraviolet absorption). In the prophase they appear to be embedded in the chromomeres, protecting the specific groups during the cleavage of the chromosomes. When their task is done, most of the nucleic acids migrate from the chromomeres to the matrix of the chromosomes. As a result, the latter absorbs stain to full extent and the chromonemata thus remain invisible during the metaphase and the anaphase; in this stage, therefore, nothing at all can be known of their exact morphology; indeed, their very existence is called into question. In the telophase the nucleic acids are for the most part degraded again. The chromosomes again become transparent and it can be seen how the chromonemata, losing their stainability, uncoil (HEITZ, 1935, p. 419) and disappear in the invisible nuclear frame.

In the view set forth here, the nucleic acids play a passive part in heredity, in that, although they protect the genes, they do not participate in their spontaneous propagation. By contrast, on the analogy of the ferments with nucleotids as prosthetic groups, an active part may be assigned to them. CASPERSSON (1941), applying his ultraviolet absorption method, discovered that vigorous protein synthesis is initiated wherever nucleic acids appear; notably that histones are formed as the result of the reaction of nucleic acids of the ribose type (absorption max. at 2900 Å) and globulins from that of the nucleic acids of the desoxyribose type (absorption max. at 2800 Å). CASPERSSON therefore declares nucleic acids to be necessary to any and every biological synthesis of proteins. According to this theory, nucleic acids would be also temporarily necessary in endomitotic division (GEITLER, 1940; BERGER, 1941), though hitherto this has evaded observation; nor can it be seen how the anucleal parts of the chromosomes augment their protein substance during division. In whatever way the function of the nucleic acids as synthesizing protein enzymes may be confirmed or modified in the future, it will not irreconcilably contradict the propounded hypothesis of screening, as in both cases nucleic acids must be assumed to accumulate in the active regions of the genes, as a result of which their chemical activity is, for the time of multiplication, paralyzed.

SCHULTZ (1941) goes one step further and calls the *genes* nucleoproteins, that is to say nucleic acid compounds. He declares that the genes and nucleoproteins have in common the properties of specificity, auto-reproduction, similar distribution in the cell and intimate relation to constructive processes. There is this much to be advanced against this opinion: that the activity of the genes only begins in the reconstituted nucleus, whereas in that state the nucleoproteins disappear very much into the background. Hence, after their duplication and division, the genes must be independent, to a large extent, of the nucleic acids, making their influence felt in the growing cell, without, having the character of nucleoproteins.

Auto-reproduction of the nucleoproteins (Comparison with virus protein). Whereas in this monograph the genes have been compared morphologically and chemically with ferments, the literature inclines rather to draw the analogy with the virus, notwithstanding the fact that important points of comparison have lost cogency since the invalidation of the classical fibrillar hypothesis pertaining to the chromosomes. Many of the varieties of virus isolated so far are of similar chemical composition as chromatin: they are *nucleoproteins*, i.e., proteins from polypeptides and nucleic acids. They do, it is true, still contain lipids and, under some circumstances, also small amounts of polysaccharides. Minute amounts of lipid have also been detected in chromosomes (HIRSCHLER, 1942), though as a rule those components are disregarded in discussions on the structure of chromatin. It is the virus of tobacco mosaic disease which has been subjected to the most exact analysis, as STANLEY's method (1938a) provides a suitable means (by precipitation) of obtaining it in a crystallized form. It contains 1.7 to 5% of nucleic acid, all according to its preceding treatment. If the nucleic acid is separated off, the virus protein loses its pathogenic properties and its propagating power. This proves beyond doubt that the mysterious auto-generation of the crystallizable viruses is determined by nucleoproteins.

There is, however, a fundamental difference as compared with the nucleoproteins of the nuclei of the cell, the virus protein showing no nucleal reaction. Thus the phosphoric compounds in the viruses are of the nucleic acid of yeast type, and not the thymonucleic acid found in the nuclei. The virus molecules are thread-like, judging by their birefringence of flow (TAKAHASHI and RAWLINS, 1933, 1935) and what the electron microscope shows us (Fig. 86). The thread molecules unite into bundles liable to grow to microscopic dimensions and then appear as crystallized virus protein. This, however, is not in a true crystalline, but rather in a mesomorphous state, for the X-ray analysis of these "crystals" produces only intramolecular interferences (BERNAL, 1939) and does not reveal any molecular lattice arrangement of the virus molecules (WYCKOFF and COREY, 1936). Thus, like liquid crystals, the parallelized thread molecules are free to revolve and shift individually.

The structure of the mesomorphous virus rodlets, which is reminiscent of that of the chromonema, favours their cleavability. On the other hand the reduplication of the chromomeres can hardly be understood as a mere splitting of bundles of parallelized molecules. The comparison is also prejudiced by our complete ignorance as to how the nucleic acids are distributed in the submicroscopically visible virus molecule. The analogy rests merely upon the common filiform structure.

It is the mysterious auto-reproduction of the virus protein which encourages comparison with the chromonemata in the chromosomes. If only a trace of the thread molecules of tobacco mosaic virus finds its way into the cells of the tobacco leaf, they fill up completely, in an astonishingly short time, with the pathogenic protein, which becomes visible as birefringent rodlets, whereas the protein proper to the cell diminishes. Thus, when in contact with virus molecules, non-virus protein becomes virus. This phenomenon has been termed *autocatalytic reproduction* (cf. W. FREI, 1943). It is known in other compounds; for example, small amounts of trypsin are liable to change a larger amount of another compound, known as "protryp·in", into trypsin. Energy is required for the spontaneous reproduction of the virus protein and this is supplied by the living cell. There can, therefore, be no reproduction of virus outside the living cell.

It is tempting to regard the duplication of the chromonemata in mitosis likewise as autocatalytic reproduction; but we should not forget that we have simply coined a term for what is at present an inexplicable process and are still quite in the dark as to the nature of the "first step" which, through contact with the specific nucleoploteins of the chromonema, has autocatalytically to be transmuted into identical nucleoproteins.

The electron microscope shows that the rod-like shape of the tobacco mosaic virus (WYCKOFF, 1947a) is an exception. The majority of the virus species photographed by WYCKOFF (1947b) have a pronounced globular shape and agglomerate in a visible crystal lattice. The morphological analogy of chromonemata and virus, therefore, is no longer supported.

Nucleus and cytoplasm. Considered from the morphological standpoint, the secret of karyokinesis is evidently that the *specific protein groups*, which serve as substratum to the genes, have to be very carefully transmitted to the daughter cells, preferably without any reciprocal changes of position. Their individuality and specific spatial relationships were developed in the course of phylogenesis and the *cytoplasm has not the power to re-create them.* The great riddle of heredity therefore still is: How can a chromonema of such complicated submicroscopic and amicroscopic morphology that it can never be produced anew, bring forth its like from itself by longitudinal

division? This mysterious process must undoubtedly take place frequently in the giant chromosomes of the Diptera, which are bundles of similar chromonemata. It is as though the chromonemata present served, as it were, as patterns for the creation of their like. It is known from the evidence of the asymmetrical C synthesis (see p. 132) that certain configurations are able to produce essentially the same morphological forms in the amicroscopic region, but the refinements of this process and its mechanism are a mystery. For here, as contrasted with the mode of action of the enzymes, it is not merely a question of fitting a key to a lock, but of how the key produces one identical to it, or the lock its exact like.

If we take the specific structures to be a given fact, we can come to an important decision as to the morphological signification of the nucleus. The genes-bearing protein threads, are in a sense self-contained and irretrievable structures and it therefore becomes clear why they are not carried along by the cytoplasmic stream, but are localised at a given spot. There they are withdrawn from the turbulent activity of the cell and perform their directive and formative task as static poles.

It is evident from the *heredity of cytoplasm* (WETTSTEIN, 1937) that specific groups must also occur in it. These special structures, however, are not solitary, for parts of the cytoplasm are similar in their behaviour to the whole cytoplast. Even fragments of the eggs of sea-urchin without a nucleus are liable to go through a certain process involving cell division (E. B. HARVEY, 1936). If, on the other hand, portions of chromosomes are removed from the nucleus while division is going on, the result is a serious modification of the hereditary process.

Although the cytoplasm is able to build up very complicated molecular systems, its architectural capacities are to some extent limited, for it cannot produce from itself the protein frame of nuclei and plastids. In heterotrophic organisms it even lacks the power to manufacture relatively simple elementary units, which are needed for plasmic synthesis; it is for this reason that these compounds have to be added as *vitamins* to the culture medium (SCHOPFER, 1936/37).

As a rule, all such problems are studied in their purely chemical aspect. Yet the molecules should not be considered only as chemical supporters of reaction, but also morphologically as elementary units of the high polymeric gel frame. In the cytoplasm, this molecular texture is very finely spun, labile and is involved in permanent reconstruction. In the nucleus, on the contrary, it has far greater density and a certain stability and is therefore distinct from the cytoplasm, not so much on chemical as on structural grounds.

§ 3. CHLOROPLASTS

a. *Microscopic Structure of the Chloroplasts*

According to the handbooks of SCHÜRHOFF (1924, p. 57), GUILLIERMOND, MANGENOT et PLANTEFOL (1933, p. 158), SHARP (1934) and KÜSTER (1935a, p. 288), the chloroplasts are microscopically homogeneous. They are described as hydrogels and both KÜSTER (1935a) and HOFMEISTER (1940) even incline to the view that they are in a liquid state of aggregation, though their flattened shape, above all, and also their autonomic transfiguration (SENN, 1908) would discount this view. As against KÜSTER's presentation of the matter (1935a), richly documented as it is, publications have been amassing since 1935 arguing in favour of a microscopic structure in living chloroplasts (HUBERT, 1935, p. 369; DOUTRELIGNE, 1935; HEITZ, 1936; GEITLER,

1937; WEIER, 1938). All the investigators mentioned find the chloroplasts to be finely granulated and for this reason appeal to SCHIMPER's (1885) and A. MEYER's (1883) grain theory. SCHIMPER's doctrine states that the chloroplasts consist of a colourless stroma, in which minute granules, lying on the boundary of microscopic visibility, are embedded; and these contain the green pigment. Colloid research, however, had utterly refuted this view, for the methods employed by colloid optics seemed to show that *all living components* of the cells are fluid (KÜSTER, 1935a, p. 290), optically empty (GUILLIERMOND, 1930) and microscopically homogeneous. Consequently, any kind of microstructure made visible in some way or other was said to be a form of precipitation, structure of coagulation, artificial product or artefact. The granular structure of chloroplasts suffered the same fate.

Photographs taken of living cells provided the evidence for the refutation of the theory that the grains in chloroplasts are a product of precipitation. The first microphotographic document of this nature may be said to have come from HEITZ (1932), who photographed chlorophyll grains next to a living nucleus in the leaf stem of *Victoria regia*. DOUTRELIGNE considers photography in red light an especially suitable means of proving beyong doubt the inhomogeneous distribution of chlorophyll in the plastids. Her objects are mosses (*Mnium*), *Vallisneria*, *Cabomba* and *Myriophyllum*. WIELER (1936) identifies the grains in a variety of *Selaginella*. But the most detailed work is undoubtedly that of HEITZ (1936), which contains microphotographs of a great number and variety of plants. The grains are decidedly identified in mosses (*Physcomitrium*, *Hypnum*, *Mnium*, *Funaria*), vascular cryptogams, very many Monocotyledones and Dicotyledones. Most authors preferred single-layer leaves, such as mosses and fern prothallia for their observations and DOUTRELIGNE avoids even the source of error involved in the use of an embedding medium, using transparent water-plants. HEITZ disdains this precaution and includes sections of living tissue in his investigations. One of the things he notices in the leaf of *Agapanthus umbellatus* is that certain chloroplasts are liable to be damaged (though the cause is not known) and in that state their granular structure is far more clearly apparent than in the undamaged specimens. Evidently this is a kindred case to the fixation of the nuclei, where a barely visible structure in the live state is coarsened in death and the blurred outlines of the optically merging structural components become more sharply defined. Seeing that so many observers have described the plastids as microscopically homogeneous, we are compelled to assume that the grana are often submicroscopic and only become visible by coarsening. Experience of nuclear structures would seem to imply that, again, it is not a matter of artefacts in this case, but rather of pre-formed structures which, lying below microscopic resolving power, or exhibiting no optically demonstrable phase boundaries, have become visible. The second alternative at the same time shows why the chloroplasts appear to be optically empty in the ultramicroscope (GUILLIERMOND, 1930).

HEITZ declares that the grana vary in size from 0.5 to 2 μ and that the size is specific to the species. As against this, observers of *Lithops* and *Mikania* state that large grana are apt to disintegrate into little ones and, conversely, small grana will form larger agglomerations. The granules in light plants are always found to be smaller than in shade plants; accordingly, the granular size increases from the upper side of the leaf (palisades) towards the underneath (spongy tissue). The grana are especially large and distinct in the chloroplasts of the green fruit of *Polygonatum* (MENKE, 1934a, who, however, calls them artificial products; WEBER, 1936).

The evidence that the grana are not globules, but platelets, is important (HEITZ, 1936b). In the side view of the flat discs of chloroplasts they look like dense streaks (cf. Fig. 120b). The HEITZ microphotographs reveal no localisation of the grana in the periphery; this conflicts with the observations made by PRIESTLEY and IRVING (1907), ZIRKLE (1926) and WIELER (1936), according to which the colouring matter is accumulated in the cortex and is lacking in the centre.

As only the grana contain the green pigment, they are the sole bearers of the fluorescence of chlorophyll (HEITZ, 1936b). METZNER (1937) was able to exhibit the grana in living chloroplasts in a wonderful way by means of an ingenious fluorescence microscope with episcopic illumination. They show up a dark red, whereas the stroma remains dark. In this way the heterogeneous distribution of the chlorophyll can be proved indubitably, even in what appears to be optically homogeneous chloroplasts.

MOMMAERTS (1938) is of opinion that the minute green particles occurring in infusions of ground leaves (NOACK, 1927) are isolated grana, which he subjects to chemical analysis. GRANICK (1938) and MENKE (1938b), however, succeeded in obtaining undamaged chloroplasts from the leaves for chemical examination.

b. *Molecular Compounds of Chloroplasts*

Proteins, lipids and the pigments chlorophyll a, chlorophyll b, as also carotene and xanthophyll, which are given the collective name of carotenoids, go to the making of the chloroplasts. MENKE finds 56.4 % of protein and 31.9 % of lipids in the chloroplasts of spinach leaves. They are rich is ash (7.8 %) and contain about 7.7 % of chlorophyll (MENKE, 1940b). Half the lipids consist of fats, 20 % of sterines, 16 % of raw wax and 2–7 % of phosphatides (MENKE and JACOB, 1942).

The proteins are localised mainly in the stroma. Cytologists call them, like the skeleton protein, plastin. Little is known of the structure of these proteins, which are fairly resistant to hydrolysis and digestion, but, on the analogy of other resistant proteins, they may be classified with the fundamental type of polypeptide chains, an assumption favoured in particular by the stringiness of the plastid substance which KÜSTER (1935c) describes. Nevertheless, the bonds between the side chains must be very loose so that the polypeptide chains can glide over and past each other. This would explain the apparently fluid state of the stroma. However, the chains cannot possess entire freedom of movement as in a true fluid, but, in the undisturbed state, they occupy definite mutual positions. This is mainly disclosed by the shape of the chloroplasts, which is unvarying and different from the droplet appearance.

There is no intrinsic chemical difference between the chloroplastic protein and cytoplasmic protein of spinach (NOACK and TIMM, 1942; TIMM, 1942); the former contains a little more histidine and somewhat less lysine and glutamic acid. According to NOACK (1930), the catalytically active iron (NOACK and LIEBICH, 1941; LIEBICH, 1941) is bound by adsorption in the stroma. It should therefore be thought of as thus bound to active side chains of the amicroscopic polypeptide frame. MOMMAERTS was inclined to view the grana as the containers of the iron, but the grana he used for his work were not perfectly pure.

Microchemically, the lipid content of the stroma has been definitely proved both by the myelin forms produced by WEBER (1933) and MENKE (1934a) from chloroplasts, and by the vital staining of the grana by the lipid dye rhodamine B discovered by STRUGGER (1936).

The formation of myelin depends upon the following two conditions: firstly the lipid molecules must be liberated from any loose linkage there may be to the protein frame so that they can "coalesce"; secondly, they must possess not only lipophilic, but also hydrophilic end groups which, as seen on p. 39, recede from each other upon the infiltration of water. The presence of water does not suffice for the formation of myelin, from which fact one may infer that the lipids in the chloroplasts have no *free* hydrophilic groups, but that these are screened off. If, however, they are liberated by saponification in a slightly alkaline medium (NH_4OH), myelin is formed at once.

We have fewer exact data respecting the chemical constitution of the grana. If they do not serve merely as energy traps, but are at the same time the loci of CO_2 assimilation, they must contain proteins in addition to pigments. EULER, BERGMANN and HELLSTRÖM are of opinion that this system is ten to twenty times the size of a chlorophyll molecule. MESTRE (1930) calls the compound between chlorophyll, lipoid and protein the "phyllochlorine complex". STOLL, borrowing WILLSTÄTTER's nomenclature, called the hypothetic compound "Chloroplastin simplex". (It should be noted that in this term the word "plastin" does not cover the sense in which the older authors employed it; they used it to denote the stroma protein, whereas it is here applied to the grana protein.) STOLL and WIEDEMANN (1941) succeeded in producing this protein containing chlorophyll in its pure state. They call the resulting chromoprotein "*chloroplastin*". Its molecular weight in the ultracentrifuge was found to be roughly five million. This compound was obtained from thirty different plant species; it does show as the haemoglobins of various vertebrata, slight differences, all according to the plant species. The chloroplastin of *Aspidistra* contains about 69 % of protein (plastin), 21 % of lipids and 8 % of pigments, 6 % of which, approximately, is chlorophyll. MENKE (1940b), finding 7–8 % of chlorophyll in toto in the chloroplasts, doubts whether the chloroplastin contains a pure chromoprotein. As, however, the chloroplastin is free from iron, it may nevertheless be assumed that it contains no essential constituents of the stroma.

We are better informed as to the structure of the *pigments* in chloroplasts than on the molecular structure of the protein. One reason for this is that the pigments are easier to isolate, another being that they are of considerable physiological interest.

The *chlorophyll* molecule is like a tadpole in appearance, having a large head and a long tail (Fig. 118). The head consists of four rings of pyrrole linked together to form one porphin ring. This harbours a magnesium atom in the centre and at its periphery are, in chlorophyll a, four methyl, one ethyl and one vinyl groups and also three oxygenic side chains, viz., one butyric acid, one acetic acid and one formaldehyde residue. The two latter are interconnected laterally (shown by 9 and 10 in Fig. 118); an isocyclic ring is therefore formed, to which has been ascribed the process of assimilation on account of its labile acetic acid-ester configuration (FISCHER, 1935; STOLL, 1936). The acid groups are esterified with methanol and phytol ($C_{20}H_{39}OH$). Chlorophyll b differs from chlorophyll a merely by the substitution of the methyl group at the 3. C atom, shown by a circle in Fig. 118, by a formaldehyde residue $-CH = O$.

There are ten double bonds in the polycyclic ring; they are conjugated, which means to say that they alternate regularly with simple bonds. Systems of conjugated double bonds like this cause absorption of light in short-wave light. Strong absorption in long-wave red is furthermore induced by the effect of porphin ring formation upon the system of unsaturated bonds. The presence of magnesium only slightly shifts the position of the various absorption bands of this system, but it does affect their intensity. It is therefore responsible for the green colour of chlorophyll. If the magnesium is removed from the porphin nucleus, the brilliant colouring fades and changes to a dirty olive brown (phaeophorbids). The slight morphological difference as between chlorophyll b and chloro-

phyll a suffices to change the bluish green colour which distinguishes chlorophyll a by increased absorption in the blue, to a yellowish green shade.

The head of the chlorophyll molecule has a hydrophilic character owing to the nitrogen atoms of the four pyrrole rings and the co-ordinately bound magnesium. Its long phytol tail, on the other hand, is lipophilic; there is, therefore, in this pigment a clavate molecule with a pronounced lipophilic pole and a lipophobic pole.

By contrast, the *carotenes*, which are unsaturated hydrocarbons of the empiric formula $C_{40}H_{56}$,

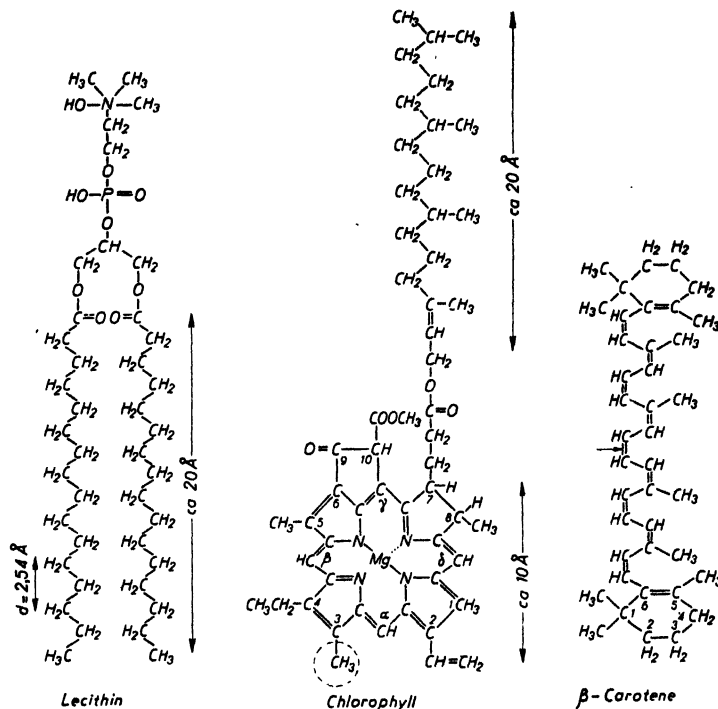



Fig. 118. Molecular units of the lipidic phase in the chloroplast (it should be noted that it is not certain wether the carotenes possess the trans configuration or the cis configuration drawn here).

are completely lipophilic. The xanthophylls, on the contrary, may contain as many as six hydroxyl groups and are therefore not so decidedly hydrophobic. Whereas it was formerly held that the carotenoids are dissolved in the chloroplastic lipids, MENKE (1940c) is of opinion that, like chlorophyll, they combine with protein molecules to form chromoproteins. An argument against this hypothesis is provided by the fact that, unlike xanthophyll and chlorophyll, carotene can be extracted by benzene and other organic solvents from dry leaf powder without any preliminary chemical action.

The structural formula of the *β-carotene* contained in the leaves is represented in Fig. 118. It is a chain of conjugated double bonds which causes the blue absorption and, therefore, the yellow-to-orange colour; it has methylic lateral groups and two terminal rings of six members. The constitution of β-carotene is a matter of paramount importance to vitamin research, for the break-down of the double bond occupying a middle position in the chain (shown by → in Fig. 118) and addition of water produce two molecules of vitamin A (KARRER). β-Carotene is optically inactive. In the α-carotene in carrot roots and in palm oil the double bond between the C atoms marked in Fig. 118 5 and 6 in one of the six-membered rings is shifted to a place between atoms 4 and 5; as a result, the C atom marked 6 becomes asymmetrical and the molecule optically active. In the case of γ-carotene the six-membered ring is open, the bond between C atoms 1 and 6 lacking. Small to larger quantities of α- and γ-carotene are often present in leaves, as, for example, α-carotene in the leaf of *Daucus Carota* (MACKINNEY and MILNER, 1933) and γ-carotene in *Cuscuta salina* (SPOEHR, 1935, p. 193). To these three carotenes may be added lycopene and others, all of which are distinct from each other by virtue

of their melting points and absorption spectra (SMITH, 1936). Like β-carotene, α-carotene and γ-carotene are pro-vitamins for the growth factor A, but they have only half its effect. This is because the two symmetrical halves of β-carotene have exactly the same chemical constitution as vitamin A, whereas, owing to the merely slight morphological changes to one of the terminal six-membered rings of α- and γ-carotene, only the unchanged half of the structural formula can produce vitamin molecules. With lycopin both the terminal six-membered rings are open, which is why this carotenoid, known chiefly in tomato, has no vitamin A activity at all. All this was discovered and presented with admirable clarity by KARRER (1935) and KUHN. It illustrates most aptly the powerful influence of the special morphology of the molecules upon the specific reactions in the organism.

There are also numerous yellow *xanthophylls* $C_{40}H_{56-n}(OH)_n$. Except for the introduction of OH groups at certain places in the structural formula, their molecules are built up in the same way as the orange carotenes. Cryptoxanthin possesses one of these hydroxyl groups at the C atom marked 3, whereas in the zeaxanthin from the grains of maize both six membered rings are substituted in this way. There are small amounts of both compounds in leaf xanthophyll, though it mainly consists of another xanthophyll with two OH groups viz., lutein, which has been known for some time from egg yolk. It comprises 50–60% of the xanthophyll (SPOEHR, 1935) in the leaves of spinach, gourd, sunflower, lettuce, barley and other leaves. The OH groups cause the beginning of light absorption to shift somewhat towards the shorter wavelengths as compared to β-carotene. In carotenoids with three and more oxygen atoms, epioxide-bridges have been discovered (KARRER, 1946).

According to the foregoing considerations, the fundamental principle of the molecular structure of all carotenoids is a relatively short chain of unsaturated hydrocarbon with conjugated double bonds. Minor variations in this type of structure give rise to the numerous carotenoids and hydroxyl substitution produces the various xanthophylls (SMITH, 1937).

As opposed to this variability on the part of the yellow pigments, we have the two green pigments, chlorophyll a and b, with their strikingly unvarying constitution. Thanks to this, the percentage of the two chlorophyll pigments contained in leaves can be determined by the quantitative method of spectral analysis (HEIERLE, 1935; SPRECHER, HEIERLE and ALMASI, 1935). The yellow leaf pigments lend themselves to such analysis only if they are composed of β-carotene and lutein and nothing else. By this method HEIERLE (1935) finds for Amersfoort tobacco at the end of July, for instance, per square metre of leaf surface: chlorophyll a 147.5 mg., chlorophyll b 53.8 mg, carotene 37.2 mg and xanthophyll 17.8 mg. This represents the familiar molecular ratio of 3:1 for the green pigments and roughly one molecule of carotenoids to every two chlorophyll molecules (about 1/3 molecule of carotene and 2/3 molecule of xanthophyll). By means of chromatographic adsorption SEYBOLD (1941) made comparative measurements and found that the molar ratios just given do not invariably exist between the pigments; chlorophyll b, for instance, may be present in far smaller quantities, or may not occur at all, this applying notably to certain algae (SEYBOLD, EGLE and HÜLSBRUCK, 1941).

c. *Submicroscopic Structure of the Chloroplasts*

We have now to synthesize the chloroplast from the molecular elementary units given and, as with most investigations in the submicroscopic field, we have to resort mainly to indirect methods. Although the ultra microscope fails us here because the plastids are optically empty (GUILLIERMOND, 1930), the chloroplasts still have three different optical effects which are measurable and which enable us to make certain inferences as to their submicroscopic structure, viz., fluorescence, light absorption and birefringence.

The last-named property owes its existence primarily to the lipoid content of the chloroplasts, while the first two provide us with information respecting the condition of the chlorophyll in the plastid.

Fluorescence. Granular chlorophyll and molecular chlorophyll solutions (in acetone, alcohol, etc.; Fig. 119a) show red fluorescence when exposed to light rays; the fluorescence is proportional to the intensity but independent of the wavelength of the incident light (WASSINK, VERMEULEN, REMAN and KATZ, 1938). On the other hand, colloidal chlorophyll solutions do not fluoresce; they can be obtained by the

dilution of molecular solutions *with water*. The chlorophyll molecules then assemble, on account of their partial hydrophobic bias, to form submicroscopic droplets (Fig. 119b). Then they lose their fluorescence, obviously because, owing to their association, the molecules reciprocally influence each other. NOACK (1927) was thus able to show that, contrary to earlier ideas, the chlorophyll cannot be present in a colloidal state in the plastids. The fluorescence persists, however, if the chlorophyll is adsorbed in a monomolecular layer to aluminium hydroxide or globulin. With NOACK, therefore, we may conclude that, in the molecular state, the chlorophyll is present in the plastids as *monomolecular films*. Fluorescence is heightened if a likewise monomolecular layer of lecithin is interposed between the chlorophyll and the adsorbant. The assumption must be that this makes the chlorophyll molecules yet more independent of each other and that there is even less mutual interference in their fluorescence. HUBERT (1935) devised a scheme by which the molecular morphology of this phenomenon is clarified (Fig. 119c). The porphin ring of the chlorophyll is orientated with respect to the hydrophilic adsorbant, whereas the hydrophobic phytol tail stands perpendicularly away from it, making the porphin system in Fig. 119 visible in profile. In this state the chlorophyll molecule may best be compared to a signet, the phytol chain being the stem or handle, and the porphin ring the seal.

Fig. 119. Chlorophyll molecule. *a*) Molecular dispersion; *b*) colloid particles; *c*) adsorbed at the lecithin layer.

As a counter to these established facts, K. P. MEYER (1939) states that his colloidal chlorophyll solutions do fluoresce; but his method of extraction is such that the chlorophyll, instead of being isolated, is dispersed in its natural association with protein and any lipids there may be in the colloidal solutions. In attempts to produce multimolecular films from chlorophyll, globulin and lecithin, NICOLAI and WEURMAN (1938) obtained non-fluorescing systems of layers.

Light absorption. The state of the chlorophyll in the living plastids may furthermore be revealed by the position of its absorption bands. For this work the BAAS-BECKING school favoured light absorption in red. Living foliage exhibits an absorption maximum at 6810 Å (BAAS BECKING and KONING, 1934; HUBERT, 1935). But this absorption band in chlorophyll isolated from the plant shifts in a varying degree towards the region of the shorter wavelengths. The effect is most marked in hexane, where the displacement amounts to nearly 200 Å, for WAKKIE (1935) finds the absorption maximum in this solvent at 6620 Å. This faces us with the task of seeking states of the chlorophyll in which its absorption comes nearest to that of the living plastids, which would permit us to predict how it will behave in the chloroplast.

According to KUNDT's law, the position of the absorption bands is governed by the refractive index of the solvent, in the sense that the higher the refractive power, the nearer will the bands shift towards the long-wave region. This, however, applies only to a limited extent to chlorophyll, viz., only in so far as solvents of equal

chemical value are compared. Thus WAKKIE finds four different series of substances to which KUNDT's law applies; they are: 1. Purely lipidic liquids like heptane, carbon tetrachloride, benzene. 2. Ethyl ether and ketones. 3. Alcohols. 4. Water, glycerol. In the lipidic solvents the red absorption band is shifted farthest from its natural position towards yellow, in the ketones somewhat less so (e.g., acetone 6640 Å) and in the alcohols still less so (ethyl alcohol 6665 Å, benzyl alcohol 6720 Å). Hence, the more hydrophilic the solvents, the closer is the approach to natural conditions in the leaf. The position of the absorption bands cannot, therefore, be improved by adding lipids (Na oleate) to alcoholic solutions; on the contrary, it is worsened by 20 Å. Solutions in water most nearly approximate the natural green of leaves (6720 Å); despite the fact that the chlorophyll is dissolved colloidally, and not molecularly, in this lipophobic solvent, the effect of the increased hydrophilic bias is to strengthen the resemblance to the conditions existing in the living chloroplast. Since it does not seem possible to find solvents in which chlorophyll displays the same absorption maximum as in the leaf, it must be assumed that the chlorophyll is not dissolved, but chemically combined in the chloroplast (STOLL's chloroplastin). K. P. MEYER (1939), who obtained fluorescent extracts with the correct absorption maximum from leaves, evidently succeeded in liberating this not very stable chlorophyll compound, undamaged, from the chloroplast; it yields colloidal solutions, in accordance with its high molecular weight. (Cp. SEYBOLD and EGLE, 1940).

Birefringence. Very important criteria are supplied by the birefringence of the chloroplasts and phaeoplasts. It was discovered by SCARTH (1924) and was found to be widespread by KÜSTER (1933, 1935b), MENKE (1934a, b, 1943), ULLRICH (1936a) and WEBER (1937). The WEBER school rightly ascribes the optical anisotropy to the lipidic substances, which can be made to emigrate; they then produce striking birefringent myelin forms (WEBER, 1933; MENKE, 1934a).

KÜSTER (1933) and MENKE (1934b) discovered the lamelliform chloroplasts of *Mougeotia*, *Mesocarpus*, *Spirotaenia*, *Spirogyra* and other algae to be clearly birefringent in profile and in cross-section, viz., negative with reference to the thickness of the plastids; the top view is, on the contrary, isotropic. Given these facts, either the entire chloroplasts, or the single grana must be optically uniaxial with negative birefringence.

MENKE (1934b) studied *Closterium* and his observations are of fundamental value. By means of reversible imbibition tests with glycerol he was able to show that the negative birefringence is due to textural anisotropy, for the birefringence drops to nil as the concentration of the imbibition liquid increases and even becomes positive in glycerol of low water content with 1.4 refractive power. This proves that the

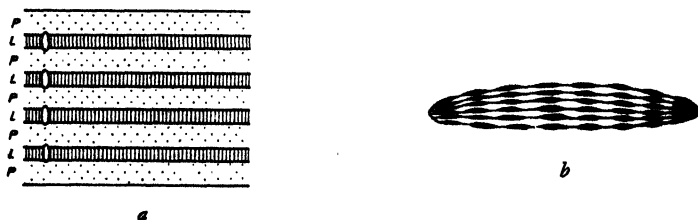



Fig. 120. Structure of chloroplasts. *a*) Submicroscopic layer structure neglecting the grana structure. P protein layer, L lipid layer, with indication of the optical character (according to FREY-WYSSLING 1937*c*); *b*) Scheme of a cross section of a chloroplast in ultraviolet light (according to MENKE 1940*d*).

chloroplasts of *Closteria* exhibit a negative textural birefringence and a positive intrinsic birefringence. It was possible to follow the rising branch of the birefringence curve of plastids fixed with osmium and formalin, up to the zero point, but not the descending branch which, after the reversal point, should return from the positive to the negative region of birefringence (MENKE, 1943). Negative textural birefringence is caused by submicroscopic platelet texture, as represented in Fig. 120. The positive intrinsic birefringence, which remains when the platelet

Fig. 121. Cross section of a chloroplast of *Anthoceros spec.* in the ultraviolet light microscope. Image scale 4000 : 1 (according to MENKE 1940*d*).

effect is suspended by the infiltration of glycerol, must be ascribed to the optically anisotropic components of the plastids, i.e., the lipids. In MENKE's experiments (1934 b) they produce myelin forms which, like lecithin, sodium oleate, etc., are as a rule optically positive with reference to the radius of the tubes. From this it follows that the orientation of the lipids in the plastids must be as shown in Fig. 120a (L). The intermediate layers are to be considered hydrophilic and it is into those layers that the glycerol must have penetrated in the imbibition experiment.

Lamellar texture. At first, MENKE (1938c) regarded my scheme (1937c) of the lamellar microtexture of chloroplasts with some scepticism. However, his labours to solve this problem led to an endorsement of my interpretation of the optical data. Gold-stained chloroplasts in profile clearly exhibited dichroism (cf. p. 70), which is indicate of a platelet texture (MENKE and KÜSTER, 1938).

The direct proof of the lamellar texture was provided by the large chloroplasts of *Anthoceros*, that classical object which, at the instigation of ERNST, had already been appealed to so fruitfully in the dispute respecting the relationship between plastids and chondriosomes (SCHERRER, 1914). MENKE and KOYDL identified layers at the limit of microscopic resolving power in cross-sections through the chloroplasts of *Anthoceros*, which exhibit no grana. The enhanced resolving power of the ultraviolet microscope revealed the picture reproduced in Fig. 121, in which the lamellar texture is clearly discernible.

Fig. 122. Edge of a chloroplast of *Nicotiana tabacum* in the electron microscope. Large and small discs lie on top of each other. Image scale 10000 : 1 (according to KAUSCHE and RUSKA, 1940).

The grana in granulated chloroplasts (*Selaginella*, *Phaseolus*) appear as differentiations, resembling nodules, of the thin lamellae, which induced MENKE to devise the plan of Fig. 120b of a section through the discoid chloroplasts of the higher plants.

Crushed chloroplasts in the electron microscope produce dislike images 10 to 20 mμ thick (KAUSCHE and RUSKA, 1940; MENKE, 1940a), such discs also appearing

(Fig. 122) when the chloroplastic lipids have been extracted. For this reason MENKE calls the discs monomolecular protein layers. He thinks they are probably built up of rod-like macromolecules standing perpendicular to the lamellar plane, recognizable in the electron images as, so to speak, overturned portions of the discs. The lamellae presumably therefore have the structure of a mesophase, which would account for the varying diameter of the discs. The grana on the fragments of chloroplasts are discernible as more strongly absorbing granules. Since smaller lamellae of the diameter of the grana are often seen side by side with the large discs in the electron pictures, the grana too probably have a molecular lamellar structure. It is open to criticism whether the electron micrographs of MENKE (1940a) justify his farreaching conclusions.

Plan of the fine-structure. Serious difficulties arise when an attempt is made to introduce the molecular elementary units of chloroplasts into the submicroscopic lamellae of Fig. 120. Such an attempt was described in the first edition of this monograph, when the then available knowledge served as the basis for a design set out in diagram. Starting from Fig. 119c, the author assumed the presence of bimolecular films of lecithin and chlorophyll in the lipid layers of the chlorophyll grana. It has, however, been proved that the molecular ratio of phosphorus to chlorophyll cannot correspond to that design (BOT, 1939; MENKE, 1940b). Only a very small portion of the chloroplastic lipids are phosphatids; the rest are phytosterol and other polar lipids. MENKE (1938c, 1943) furthermore points out that chlorophyll migrating with the lipids imparts conspicuous dichroism to the myelin patterns of chloroplasts, lacking in the profile of the chloroplasts. It is therefore improbable that the chlorophyll in the chloroplast is associated with the orientated lipids.

Thus everything goes to show that the chlorophyll is combined with proteins to form a chromoprotein and that STOLL's chloroplastin theory (1936) is well founded, not only chemically, but also morphologically. To be able to form any idea of the arrangement of the chlorophyll molecules, it would be necessary to know how many of them go to every chloroplastin unit. A rough estimate produces an unexpectedly large number. The molecular weight of chloroplastin is roughly five million, whereas that of chlorophyll is only 900. Thus the stoichiometrical ratio is about 5000:1. Quantitatively, however, the chloroplasts contain only 56.4 % of protein to 7.7 % of chlorophyll, which is a ratio of only 7:1. Consequently, every chloroplastin molecule must contain 700 chlorophyll molecules combined! Regarded as globular particles, protein molecules of five million molecular weight have a diameter of approximately 100 Å (see Fig. 2). The surface of a particle of chloroplastin would, therefore, amount to π $(100)^2$ Å^2; now, as the surface of a porphin ring measures about (15^2) Å^2, only about 140 chlorophyll molecules could be bound by a chloroplastin molecule by surface adsorption. The inference is that the surface of spherical chloroplastin molecules cannot be the only place where chlorophyll is to be found, but that it must to a considerable extent be housed in the interior of the particles. We might imagine numerous side chains of folded polypeptide chains entering into relation with each chlorophyll molecule. If we may assume that each chlorophyll molecule has its own individual carrier, as is the case with the ferments (see Fig. 110, 117f), then it would mean that particles of chloroplastin comprise hundreds of such units.

As to how the large macromolecules of the chloroplastin simplex are arranged in the chloroplast, they are less likely to take up random position than to orientate

under the impulsion of the incident light energy. Seeing that, in physiologically optimum illumination, the chloroplasts automatically arrange themselves so that the light shall fall upon them perpendicularly, it is obvious that the chloroplastin units are to be conceived of as arranged in molecular layers.

If this conception of the arrangement of the chromoprotein be correct, the principle of laminar surface development can be consistently pursued from the molecular to the macroscopical region. The molecular layers of chloroplastin compose the discoid, submicroscopic to microscopic grana (Fig. 120a); these, again, lie in layers in discoid or laminar chloroplasts and finally the chlorophyll grana are exposed to the light, again in foliar laminae.

Tracing thus a given morphological principle through several orders of magnitude, we are provided with an interesting counterpart to *fibre structure*, in which linear development plays a similar part. The laminar series: molecular layer / grana / chloroplast, / foliar laminae may be compared with the linear series: chain molecule / fibrilla / fibre / fibre bundles of the pericycle. In both cases the units on the boundary of the microscopic and submicroscopic region (grana and fibrillae) are controversial values, opinions differing as to their individuality. This, however, makes no difference to the principle of structure, which holds good, regardless of the independence ascribed to those units. It should be emphasized that in both cases the textural birefringence has been the key to clearing up of the submicroscopic structure, viz., the discovery of the rodlet birefringence in fibres and of the platelet birefringence in chloroplasts.

Unit of assimilation. Whereas chemists think of the photosynthetic process as associated with the chlorophyll molecule (STOLL, 1936), physiologists tend rather to regard the pigment merely as an energy trap and to attribute the actual chemical action of the gradual hydrogenation to the proteins in the chloroplast (RABINOWITCH, 1945). This is inferred partly from BLACKMAN's dark reaction (1905), but mainly from facts established by EMERSON and ARNOLD (1932), according to which a *unit of assimilation* of roughly 2500 chlorophyll molecules is needed for the reduction of a CO_2 molecule. GAFFRON and WOHL calculate about 1000 molecules for this same unit. This observed fact calls into question all attempts to deduce the mechanism of assimilation from the chemical constitution of the chlorophyll molecule (WILLSTÄTTER, 1933; FRANCK, 1935; STOLL, 1936). GAFFRON and WOHL (1936) state that the pigment acts merely as a specific energy transmitter and that a very large number of chlorophyll molecules would be required to capture the necessary quanta of light for the assimilation of one CO_2 molecule (WARBURG and NEGELEIN, 1923; SCHMUCKER, 1930; EYMERS and WASSINK, 1938; EMERSON and LEWIS, 1939). It is to be expected that the occurrence of these units of assimilation will be expressed morphologically in some way. HEITZ (1936a) presumes that the grana may be involved. This, however, cannot be so, for if, as EULER, BERGMANN and HELLSTRÖM (1934) state, a chloroplast contains $1.65 \cdot 10^9$ chlorophyll molecules, there would have to be something like 10^6 or a million grana. In a bimolecular layer, 2000 chlorophyll molecules would occupy a surface of $1000 \cdot 225$ Å^2 $= 2.25 \cdot 10^{-8}\ \mu^2$. As a square this surface has a side of only 0.05 μ. Therefore the unit of assimilation is certainly *amicroscopical.*

To BAAS BECKING and HANSON (1937) the unit of assimilation is merely a statistical value. They suspect that four chlorophyll molecules apiece are united to form tetrads, in which the reactive iso-cyclic rings (Fig. 118, C 6, 9, 10) turn towards each other. The carbonic acid of assimilation is, they think, bound as orthocarbonic

acid and hydrogenated in the centre of symmetry of the four asymmetrical, tetragyrically arranged plane porphin nuclei. Owing to intermolecular rotations of the independent porphin rings, it is supposed that the exact juxtaposition necessary to the fixation of a CO_2 molecule occurs so rarely that only one tetrad among 1000 to 2000 chlorophyll molecules will be in the proper condition to assimilate. This theory assumes fourfold axes of rotation as elements of symmetry perpendicular to the monomolecular layers of chlorophyll, which should somehow be noticeable in the crystallized chlorophyll. No such confirmation has, however, been forthcoming so far; on the contrary, threefold screw symmetry has been found to be the principle of arrangement in ethyl chlorophyllid, which is obtainable as crystallites (KETELAAR and HANSON, 1937). Nevertheless, this hypothesis has the undoubted merit of having reinstated the earlier view that the process of assimilation takes place in contact with the chlorophyll molecules. For, on the premise of the chloroplastin theory, the functions of chlorophyll as an absorbent molecule and of the protein as the carrier of the system of hydrogenation are no longer strictly discrete, since the two components are united to form one large macromolecule. Not all the parts of a giant molecule of this kind will, of course, react in the same way; but presumably the partial functions will act harmoniously and it will not be possible to analyse them individually.

Seeing that a chloroplastin macromolecule must contain several hundred chlorophyll molecules, the question naturally arises as to whether, maybe, the unit of assimilation is identical with the chloroplastin unit, the molecular weight of which is five million. As the chloroplastic protein is divided between stroma and grana and we have only grana protein to consider for chloroplastin, the number of chlorophyll molecules calculated for every chloroplastin molecule, in the foregoing section is even too small. If, for, example, the chromoprotein contains only one-third of the total plastid protein, the number of chlorophyll molecules per chloroplastin macromolecule would mount to about 2000; then the chemical and physiological units would more or less coincide.

§ 4. ERYTHROCYTES

a. *Microscopic Structure of the Erythrocytes*

It is not only their lack of a nucleus which makes the red blood corpuscles of mammals a cytological curiosity, but it is also the peculiar shape of the cell. Seen from the top in the microscope, they look like round discs, the boundary of the cross-section of which is curiously sinuate, instead of being planoparallel. Thus the erythrocytes are *biconcave discs*. This remarkable shape of the cross-section is said to be due to the function of the red blood corpuscles, since from a surface thus shaped the interior of the cell can be easier supplied with oxygen by diffusion, whereas a globular shape would entail greater poverty of oxygen in the centre than in the surface layers and, with a planoparallel disc, the edge would be richer in oxygen than the centre.

Fig. 123. Cross section of the red cell of man. ab = 8.55 μ; thicknees ½ (cd + ef) = 2.40 μ; thickness gh = 1.02 μ (according to PONDER 1934).

The discs remain biconvex in shape as long as the erythrocytes are suspended in the blood plasma or in serum, but they round off directly if the medium is changed

by the addition of lecithin to the blood plasma. It is a remarkable fact that the same thing happens when a thin layer of them is covered with a cover class. PONDER (1934), discussing many possible causes of this phenomenon, omits to mention the change in r_H of the medium and asphyxiation, which all living cells undergo after some time in the thin layer under the cover glass. Under certain circumstances rounded blood corpuscles can be restored to their initial biconcave disc shape by the addition of serum.

As any experiment with erythrocytes involves possible transformation, it is not an easy matter to establish their true cross-sectional shape. PONDER (1934) obtained the image shown in Fig. 123 by a series of microphotographs with an objective of the least possible focal depth. With retention of the volume, the transformation to spheres is effected by surface changes only. For instance, the biconcave erythrocyte of the rabbit has a surface of $110\mu^2$, whereas that of the globular form is only $70\ \mu^2$ (reduction in surface of 36 %).

A further indication of surface changeability is provided by the dented blood corpuscles, which are transitions between the biconcave discs and the globules, or the curious thorn-apple forms which arise under certain outside conditions. These facts make it plain that surface forces are responsible for the shape of the erythrocytes. GOUGH (1924) points out that surface-enlarging forces must be active in the erythrocytes, conducive of expansion of the surface of contact with the suspension liquid, as in the case of the myelin forms. The largest surface would be obtained if the blood corpuscle were flattened to the thinnest possible disc. On the other hand, there is some slight surface tension in the blood corpuscles (presumably of the order of 1 dyne/cm, cf. Table V) tending to reduce the surface and to round off the erythrocytes if outer conditions do not interfere.

Now it may be that the exceptional shape of the erythrocytes of mammals represents some kind of equilibrium between the surface-enlarging and surface-reducing forces. If that be so, the membrane of the erythrocytes should have the properties of a mesophase. No form of equilibrium can, however, be mathematically computed from the cross-section in Fig. 123 and PONDER (1934, p. 89) therefore inclines to the belief that there must be a certain amount of internal solidity. The micrurgical investigations of SEIFRIZ (1927, 1929) tend to endorse this, for they show that deformed and elongated erythrocytes have some slight eleasticity. DERVICHIAN does not agree with this view (DERVICHIAN, FOURNET and GUINIER; 1947).

Inner structure. The various theories as to the internal structure of the red blood corpuscles are expressed in the following two views. One school regards the anucleate erythrocytes as enclosed in a membrane which gradually changes towards the interior into a very loosely knit *spongy structure*, in which the red blood pigment is embedded. Some support for this view is afforded by the network structure which can be made visible in young erythrocytes by suitable fixation and staining. Representatives of the other school of thought, however, dismiss this network as mere artefact. To their way of thinking, the erythrocyte consists merely of a *balloon-like membrane*, a view which has some backing through the absence of any microscopic structure in the living cell interior as seen in the ultra microscope or illuminated by ultraviolet rays. This view is also shared by most of the research workers who have studied *haemolysis.* For, if the erythrocytes are damaged mechanically, either by heat or freezing, or by immersion in sufficiently hypotonic or hypertonic solutions, the contents of the cell extravasate with the red blood pigment and a colourless sheath remains, which is called the stroma.

These facts notwithstanding, the contents of the erythrocytes are not to be considered as a sol-like liquid of no organised intrinsic structure, an error committed by the older investigators (SCHWANN, NORRIS) and, more recently, by GOUGH (1924). The relative viscosity of the cell contents is 30 (see Table XX) and PONDER (1934) states that the interior of the cell manifests respiration similarly to other cells. Although the erythrocyte membrane has been proved to contain so to speak all the chemically identifiable substances of the blood corpuscles with exception of the blood pigment and the salt content, the assumption clearly must be that the contents of the cell, far from being an unorganised vacuole liquid, is a fluid cytoplasma, the organisation of which is easily destroyed when damage is inflicted.

b. *Molecular Compounds of the Erythrocytes*

Erythrocytes consist approximately of two-thirds water and one-third dry residue, which is mainly composed of the red blood pigment, haemoglobin, and salts. It is interesting to note that potassium predominates over sodium as cation of the salts. Small amounts of protein foreign to haemoglobin and of lipids constitute the erythrocyte membrane.

Haemoglobin. The red blood pigment is a chromoprotein, like chloroplastin in green leaves; yet the link between chromogen and protein is closer than in chlorophyll and the blood pigment therefore emerges as protein from the stroma in haemolysis.

Haemochromogen is a labile porphyrin compound which, outside the organism, is transformed into the more stable haematin. The composition of this compound is as follows: $C_{34}H_{32}O_4N_4FeCl$ and it is closely akin to chlorophyll. The main differences are that in the centre of the porphin ring there is, instead of magnesium, trivalent iron, the third valency of which imparts a saline nature to the compound usually neutralized by the anion chlorine; and the absence both of the phytol chain and the iso-cyclic ring of the C atoms 6–9–10 (see Fig. 118). As a result of the missing phytol chain the haematin appears to be morphologically more compressed and less markedly polar than chlorophyll. The protein carrier, to which the haemochromogen is attached is called "globin".

The haemoglobin molecule is globular or of thickset rod-like shape. On the basis of the iron content its molecular weight is computed at 16000 to 17000 (KARRER, 1941), while the reading in the ultracentrifuge is 69000, i.e., about four times the value (SVEDBERG's law of multiples, see p. 216).

Stromatin. JORPES (1932) states that approximately 4 per cent of the total protein content of the erythrocytes consists of a protein foreign to haemoglobin, which is contained in the erythrocyte sheath and is therefore described as stromatin. According to WINKLER and BUNGENBERG DE JONG (1941), its I. E. P. is at p_H 5.2. Analysis of the haemolysed membrane of erythrocytes shows that there is 80 % of stromatin and 20 % of lipids.

Phospholipids. The bulk of the lipids consist of phosphatides, notably lecithin (Fig. 118), besides which there are insignificant amounts of cephalin and sphingomyelin. The I. E. P. of the phospholipids is at p_H 2.7. They are thought to play a decisive part in the permeability phenomena of the red blood corpuscles.

Cholesterol. Approximately one molecule of cholesterol is found to every four phosphatide molecules in the stroma (exact ratio 3.5 : 1, WINKLER and BUNGENBERG DE JONG, 1941). As may be seen in Fig. 92, cholesterol, contrary to the phosphatides,

possesses no ionogenic groups. BUNGENBERG DE JONG therefore assigns to it an important part in the formation and build-up of lipid structures, for, in a lecithin solution, the individual lipid molecules remain separated from each other as the result of their negative charge. True, the fatty acid chains have a tendency to agglomerate, but the repellent effect of the ionized phosphoric acid groups predominates and the molecules are therefore kept at a distance from each other. If cholesterol is added to a solution of this kind, the short-rod, neutral molecules are able to penetrate in between the lecithin molecules and association follows as the result of VAN DER WAALS cohesive forces, as the repelling action of the charges does not span the width of the cholesterol molecule. Cholesterol therefore acts as a *sensitizer* in the precipitation of lipid solutions with ionogenic groups. Conversely, in lipid films of phosphatides, cholesterol acts as a *stabilizer*, as it counteracts solution of the film by ionogenic influences.

c. *Submicroscopic Structure of the Membrane of Erythrocytes*

Stromatin as tricomplex system. WINKLER and BUNGENBERG DE JONG (1941) have published an instructive design of the structure of the erythrocyte sheath (Fig. 124). By exact measurement of the electric migration velocity of the red blood corpuscles in the most various salt solutions, these investigators find quantitatively the same behaviour as in phosphatides, from which they conclude that the surface of the erythrocytes is covered by a phosphatid film (layer I in Fig. 124), which is stabilized by cholesterol. The I. E. P. of the stroma with p_H 5.2 being between that of the phospholipids (2.7) and of the stromatin (5.8), it is assumed that the phospholipids form a complex system with the stromatin (layer IV), their positive choline groups entering into relationship with the anionic end groups of the protein (layer III). Haemolysis experiments have further shown how calcium ions consolidate the erythrocyte membrane and stabilize it. In layer II the calcium ions, with their strong positive charge, are therefore allocated between the negative phosphoric acid groups of the lecithin and a more powerful ionogenic cohesion is thereby attained. Thus the stroma is regarded as a complex system consisting of phosphatid-calcium ions, stromatin protein, and the regular distribution of charge brings with it a definite arrangement and orientation of the various components of the system.

Fig. 124. Molecular structure of the envelope of the red cell according to WINKLER and BUNGENBERG DE JONG (1940-41); • anionic groups; o kationic groups or kations (Ca); shaded: cholesterin; z phospholipid acid; •——— fatty acid.

The tricomplex system is completed by the assumed complex linkage of the haemoglobin (layer VI) with anionic end groups in layer V to cationic groups of the stromatin. The presumed binding of the haemoglobin receives support from the spectroscopic investigations of ADAMS (1938), who found that the 4100 Å absorption band of the red blood pigment was missing

from the erythrocytes, which shows that dissolved haemoglobin is not present in the interior of the cell.

The design of Fig. 124 is further complemented by layer A. This represents an incomplete film of polar lipids, which turn their lipophilic side towards the monomolecular phosphatid layer I and their hydrophilic pole outwards (fat, fatty, acids, possibly cholesterol). It is necessary to assume this, as, without this layer A, the erythrocytes would agglutinate in aqueous solutions and, when shaken out with paraffin oil, would pass over into the lipid phase, neither of which they do.

The scheme devised by WINKLER and BUNGENBERG DE JONG (1941) explains many properties of the erythrocytes. E.g., it makes allowance for the lipid filter theory of permeability, there being a lipid film with molecular pores (where the cholesterin covering is lacking). It explains the permeability to anions which is characteristic of erythrocytes, as the calcium ion layer III debars the cations. The same layer of ions, with its water of hydration, is responsible for the effect of hydrating and dehydrating ions upon the properties of the erythrocytes. According to FRICKE (1925), the electric properties of the wall of the erythrocytes are such that the existence must be assumed of a non-conductive layer 33 Å thick. This thickness corresponds to the lipidic part of the phosphatid layer I. GORTER and GRENDEL (1925) assume that there is a bimolecular lipid film on the basis of the lipid content of the erythrocytes; and this claim is likewise partly met.

Finally, WINKLER and BUNGENBERG DE JONG calculate from the stromatin and lipid contents of the erythrocytes of pigs (19.2, or 3.5 mg per ml of blood) that the orientated lipid molecules just cover the surface of the blood corpuscles in the manner indicated (Fig. 124) and that the layer of stromatin below is 120 Å thick. From this we get 150 Å as the thickness of the total erythrocyte membrane (without layer A) which, surprisingly, is of about the same order of magnitude as the data obtained by WOLPERS (1941) by means of electron optics (see p. 172).

Although this explanation of many interesting phenomena associated with the morphology and physiology of erythrocytes is undisputed, the model of Fig. 124 still raises a number of difficulties. One of the first points to be noted is that analysis of the erythrocytes has not revealed the presence of calcium. True, WINKLER and BUNGENBERG DE JONG have calculated that the quantity of Ca present is so small that it would escape detection in analysis, but they nevertheless consider it improbable that, given the percentage of calcium in the blood serum, no Ca ions should be adsorbed by the erythrocyte membrane. In the transition from the biconcave disc shape to the globular, the surface must shrink by 37 per cent. It is not clear this could take place without causing change of structure since, compared to their normal distances, the molecules are already densely packed.

An argument against the parallelized radial orientation of all the molecules is the slight optical anisotropy of the erythrocytes. Stromatin and haemoglobin can scarcely be said to represent chain molecules; on the contrary, haemoglobin is known rather to be a globular molecule. Should stromatin be filamentous, it would seem to me that the orientation of those threads, given their great length, is more likely to be parallel to the surface than a radial one, as suggested. WINKLER and BUNGENBERG DE JONG discuss this possibility; but, finding that the number of anionic COOH groups of the side chains is not large enough for their tricomplex system, they place the main chains perpendicular to the surface of the cell.

Optics. Fresh problems arise as soon as due regard is paid to the optics of ery-

throcytes. Rabbit's erythrocytes, carefully haemolyzed by freezing and thawing, are birefringent (SCHMITT, BEAR and PONDER, 1936, 1938), exhibiting a very faint polarisation cross. The birefringence is slightly negative in isotonic salt solution, but positive polarisation crosses are clearly visible in glycerol mixtures. The inference from imbibition tests of this kind is that, as in the case of the chloroplasts, in the sheaths of the erythrocytes there is positive intrinsic birefringence of the embedded lipids, upon which is imposed a negative layer birefringence of the protein framework. Lipid solvents, such as butyl and amyl alcohol, produce distinctly negative polarisation crosses, neutralizing the intrinsic birefringence of the lipids and bringing the negative layer birefringence out clearly.

SCHMITT, BEAR and PONDER come to the conclusion that there must be a composite body with alternating protein and lipid lamellae. The lipid layers,they think, must be bimolecular on account of the hydrophilic bias of the stromatin. This view conflicts with the calculations made by GORTER and GRENDEL (1925), according to which the lipid content of the erythrocytes would be just sufficient for a single bimolecular covering. The possible layering throughout the stroma would only be lipid-protein-cavity-protein-lipid. Consequently, unless those authors' statements are incorrect, it is difficult to see how there can be a composite body of protein and lipid, like that proved for the chloroplasts. It is, however, quite easy to imagine that the lipid does not form a continuous layer covering the erythrocytes, but that it is interspersed in the protein framework, like lenses. This kind of texture would produce the same effect of layer birefringence (cf. p. 58) and would be compatible with the various mosaic theories, which assume protein areas as well as lipid regions.

Another possible explanation, taking the observed facts into account, is that the stromatin is loosely layered and is in itself a WIENER composite body. In this case, too, the positive intrinsic birefringence of the lipid skin overlays the negative layer birefringence, the problem, however, still being whether the lipid birefringence would then be perceptible at all. The probable retardation Γ can be calculated with the aid of the formula on p. 61 by inserting the value 0.011 for the birefringence $\triangle n$, which BEAR and SCHMITT (1936) set down for orientated lipid in the nerve sheath. In rabbits, the diameter d_1 of the, supposedly, hollow cylindrical rim of erythrocytes is 1.7 μ (cf. c–d in Fig. 123), and d_2 is shorter by twice the thickness of the bimolecular lipid layer (4×3 mμ), i.e., 1.688 μ. The value for the retardation Γ is then a little above 1.8 mμ. This is a value which, though at the lower limit of quantitative mensurability with sensitive compensators, may, by suitable polarising optics, be revealed qualitatively. This shows that a single bimolecular lipid layer suffices to produce the faint positive intrinsic birefringence detected by SCHMITT, BEAR and PONDER.

Both the quantity of lipid present and the slight intrinsic birefringence witness to the fact that there can hardly be more than a double film of orientated lipid molecules in the erythrocyte. This eliminates the possibility of a protein-lipoid layer composite body, such as demonstrated in chloroplasts. To account for the lamellar birefringence, therefore, one is forced to assume that the stromatin protein is lamellar with, may be, layers of hydration in between. These need not necessarily be continuous; indeed, they are more probably like cavities shaped somewhat like lenses. On this assumption the direction in which the stromatin molecules of Fig. 124 (layer IV) are orientated must undoubtedly be turned at an angle of 90^0 and parallel to the erythrocyte surface.

Electron optics. Apart from fibres and diatoms, erythrocytes were the first cytological object to produce good and impressive images in the electron microscope (WOLPERS, 1941). This is due to their ability to withstand complete drying without

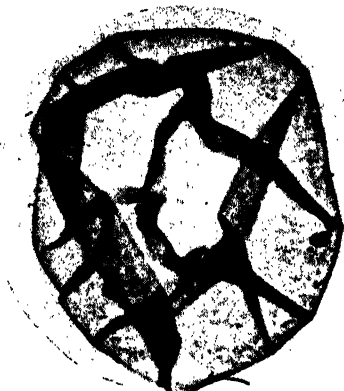
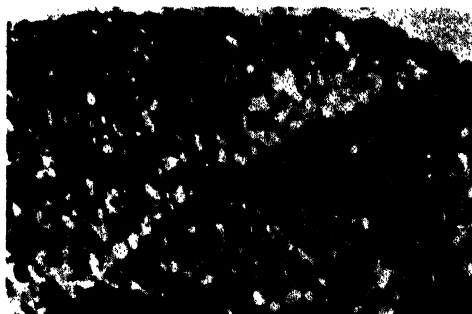



Fig. 125 Fig. 126

Fig. 125. Membrane of red cells, osmic fixation. Electron microscope 9500 : 1 (according to WOLPERS 1941). – Fig. 126. Membrane of red cell after extraction of lipids. Electron microscope 34000 : 1 (according to WOLPERS 1941).

any concomitant essential change in structure, as we know it from the fixation method with a flame.

The photographs of the residue of haemolysis (Fig. 125) merely show a folded membrane. No inner structure is visible, which WOLPERS thinks proves the balloon theory of the structure of erythrocytes. The average thickness of the membrane is 250 Å. This direct mensuration by WOLPERS discounts data obtained by indirect means which are not in agreement with it (FRICKE, 1925, 33 Å; SEIFRIZ, 1927, 7000 Å). There are also electron optical measurements of nonhaemolysed blood corpuscles, but they cannot be accepted without caution, as the contours are not absorption shadows, but interference fringes (see p. 84), which are apt to give rise to miscalculations.

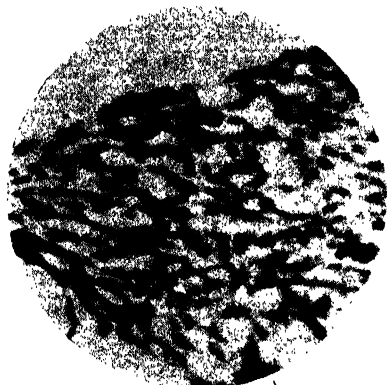



Fig. 127. Stretched membrane of red cell in electron microscope. Image scale 51000 : 1 (according to WOLPERS 1941).

After suitable extraction of the lipid, the erythrocyte membrane appears to be porous (Fig. 126). WOLPERS therefore discards as improbable the layer structure inferred from observations in the polarisation microscope. He, however, also rejects the idea, of mosaic structure, which Fig. 126 would at first seem to suggest; for he detected a network structure in stretched erythrocyte membranes which had been fixed with osmium after extraction of the lipids. This induces him to believe that the stromatin has a *frame structure*, in the meshes of which he imagines the lipids to be embedded. Whether this opinion is shared, or the meshes are thought to be free from lipids and filled with an aqueous phase, depends upon the rejection or acceptance of a superficial double film of lipid. However this may be, the optically

proved lamellar structure must not be ignored; rather should an attempt be made to reconcile the two findings.

A consistent picture is obtained if the filamentous protein frame is thought of as stratified parallel to the surface and the meshes as shallow, tangentially extended lenses, when the body of the framework exhibits layer birefringence. Under these circumstances, certainly, no pores would be visible in the top view of the skin. In any event, it will be clear from a comparison of Fig. 126 with 127 that the pores of Fig. 126 are differently constituted from the meshes of Fig. 127. The impression received is that the sieve-like image is not the natural structure, but an artefact. This suspicion is strengthened when one examines JUNG's photographs (1942) of erythrocyte membranes denatured by heat haemolysis. In them there are similar sieve images, with even larger pores. Possibly, therefore, the holes in Fig. 126 were caused by incipient denaturation in the extraction of the lipids, or maybe by the bombardment with electrons.

Putting together what we know with fair certainty of the submicroscopic intrinsic structure of the erythrocyte membrane, we must come to the conclusion that the stromatin has a micellar reticular structure which appears to be laminated on account of the tangential strand arrangement. There are lens-shaped to flat intermicellar spaces. The lipids are either embedded and orientated in these lenses, or they envelop the whole surface of the erythrocytes in a continuous double film. The quantity of lipid is too small for a protein-lipid layer body.

This exposé of the microstructure of erythrocytes demonstrates impressively the fact that submicroscopic morphology cannot be inferred from either the indirect methods, or from direct electron microscopy, alone, but that the two modes of enquiry should be complementary and the results obtained with the one should be scrutinized in the light of the data produced by the other.

III. FINE-STRUCTURE OF PROTOPLASMIC DERIVATIVES

The distinctive feature in the structure of living protoplasm is the absence of homogeneous lattice regions, whereas the intrinsic structure of protoplasmic derivatives is as a rule conditioned by the arrangement of the molecular elementary units in some lattice order. This is due to the fact that protoplasm is made up of various chain molecules and specifically different chains of polypeptides, while on the other hand the high-polymeric substances of the frame and reserve substances generally consist in the main of only one particular kind of macromolecules which combine to form an orderly pattern with comparative ease and thus lend themselves to X-ray analysis. For this reason we are much better informed on the submicroscopic structure of these metaplasmatic and alloplasmatic cell constituents than on the intrinsic structure of the living substance. It is not without significance that the revised formulation of the micellar theory — which can conveniently be applied to plasmic structure by means of the transition from the micellar to the molecular frame — derives from the structure of the cell walls. Thus, while we can only trace the intrinsic structure of plasm in general outline, we have abundant measurable data concerning the ultrastructure of the frame substances. This monograph will deal with the structures disclosed up to date and will be concerned less with the physico-chemical than with the biological questions inherent in the theory of microstructure. In the chapter on the frame substances, the macromolecular substances making up the frame are mentioned within brackets after the subtitles.

§ 1. FRAME SUBSTANCES

a. *Meristematic Plant Cell Walls (Cellulose)*

The primary cell wall. There is a very real physiological and histochemical difference between the *primary cell wall* of vegetable meristems and the secondary membranes of grown tissue. It is before all in their surface growth (intussusception) that this difference stands out, the secondary wall layers being, on the contrary, deposited by apposition against the fully-grown primary wall during the corresponding growth of the membrane in thickness. In many respects, therefore, the primary membranes behave very differently from the strong secondary walls. Above all, they lack the microscopic markings such as pits and striations, etc. They are very thin and are therefore considered as the intermediate membrane between neighbouring cells, actually consisting of three lamellae, viz., the original, odd middle lamella produced from the cell plate during cell division, and the two primary walls added on to it. Another important point about meristematic cell walls is that no cellulose can be

identified microchemically in them (TUPPER–CAREY and PRIESTLEY, 1923). GUNDERMANN, WERGIN, and HESS (1937) nevertheless detected by X-ray the fibre period of cellulose in the elongating cells of *Avena* coleoptiles (after removal of the epidermis). As their photographs show only the interferences of the lattice planes perpendicular to the chain axis, evidently the cellulose strands present consist of so few parallel chain molecules that there is an insufficient number of lattice planes parallel to the chain axis to produce X-ray interferences. THIMANN and BONNER (1933) found by analysis 42 % of cellulose in dried *Avena* coleoptiles but, just as in HEYN's X-ray investigations (1933, 1934), this percentage includes the epidermis with already thickened walls resisting cell extension. Although unthickened meristem walls contain less cellulose, they certainly contain an already cohesive, fine frame work of cellulose chains. Seeing that the cellulose is masked by other constituents of the membrane (see p. 42), it is particularly fortunate that its presence can be betrayed by its birefringence. Pectins, which are attending cellulose, have never yet been found to show birefringence in the plant.

The view I advanced (1935b) at the International Botanical Congress held at Amsterdam, to the effect that quite young meristematic cell walls already contain a submicroscopic cellulose frame (Fig. 128), was at first disputed by HESS and his co-workers, they having overlooked the birefringence of these cell walls (HESS, TROGUS, and WERGIN, 1936). Later, however, they admitted that cellulose can be identified by X-ray after cold water extraction, since, after the removal of water-soluble intermicellar substances, collective crystallization of exceedingly thin strands of cellulose takes place (HESS, KIESSIG, WERGIN and ENGEL, 1939).

Birefringence enables the investigator to detect when, during the formation of the young membrane after the division of the cell, cellulose first makes its appearance. BECKER states (1934) that the so-called cell plate in the phragmoplast of the staminal hairs of *Tradescantia* first becomes visible as droplets exhibiting a Brownian movement. They do not, he says, move along the spindle filaments, as is assumed by others, but are formed, just where they are, by dissociation from the dense plasm (BECKER, 1935). The drops adhere laterally and form a grained isotropic membrane which, however, does not at first touch the side walls and shows a pectin reaction (coloration with ruthenium red). Plasmolysis reveals its independence. From the moment this system has grown completely trough the phragmoplast and reaches the wall of the mother cell, this diaphragm becomes visible between crossed Nicols. Apparently the phragmoplast, split into two halves, immediately generates cellulose on its surface where it is in contact with the new membrane. It seems to me improbable that a cellulose frame would develop from the droplets described by BECKER. It is also difficult to understand how protopectin could be formed from liquid drops. I therefore suspect that the drops are water of hydration liberated when high-polymeric chain molecules are built up in the cell plate from sugars of low molecular weight. The fact that the microvacuoles are dyed vitally with basic dyes (neutral red) does not invalidate this view, since they may quite conceivably contain water-soluble components, though they can scarcely harbour insoluble high-polymeric material such as protopectin or cellulose. These wall substances must be formed submicroscopically in the phragmoplast and do not become visible until a microscopic system of protopectin has been built up, against which cellulose mixed up with protopectin is then immediately deposited on both sides. Hence the original middle lamella and both primary walls are already present in this very young state, but

presumably all three membranes increase in thickness before surface growth begins.

Cell elongation. The submicroscopic morphology of extension, in which short cylindrical cells multiply their length in a short space of time, is familiar. All meristem cells capable of stretching are of tubular texture, as has been demonstrated in the case of *Avena* coleoptiles (SÖDING, 1934; BONNER, 1935), of the filaments (SCHOCH-BODMER, 1936; FREY-WYSSLING, 1936c), the rapidly growing sporogonous stem of the moss *Pellia* (OVERBECK, 1934; VAN ITERSON, 1935), to mention only a few; likewise cotton hairs (WERGIN, 1937), bast fibres and all derivatives of cambium (MEEUSE, 1938, 1941) possess extremely thin, scarcely visible primary walls of tubular texture. The very delicate cellulosic framework of a wall of this kind is illustrated in diagram by Fig. 128. Another scheme of the primary cell-wall based on individual (corpuscular) cellulose particles which, to my mind, are quite impossible is given by HESS (1943). The coherent texture of interwoven micellar strands (with 150–200 Å diameter) has been demonstrated by ELVERS (1943b) with electron micrographs.

Fig. 128. Submicroscopic structural plan of the meristematic primary wall of cylindrical cells. (FREY-WYSSLING 1936a).

It should be borne in mind that with tubular texture the cell wall is negative with respect to the cell axis. When stretchable tissues are elongated by mechanical means, the birefringence of their cells changes and becomes positive; but if they stretch through growth they remain negatively birefringent. We have to ask ourselves, therefore, why the cell-wall optics of artificial and natural stretching should be opposite.

The diagram of the tubular texture (Fig. 128) can be idealized, as in Fig. 129, as a regular rhombic network. The junctions, where the long cellulose strands are in contact, have a certain firmness. Therefore all extension is at first of an elastic nature. The shifting of the frame bars involves stresses. Consequently, the least amount of elongation must produce a positive photo-elastic effect (see page 62). That is why even comparatively slight extension may cause the negative intrinsic

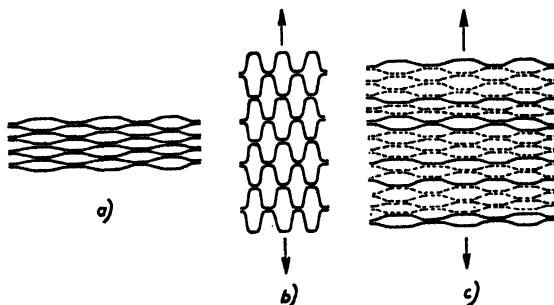



Fig. 129. Extension of the micellar frame of Fig. 128 (after FREY-WYSSLING 1945a). *a*) Idealized; no tensions. *b*) Artificial extension; junctions not detached. *c*) Stretching through surface growth; junctions loosened and new cellulose strands (dotted) embedded by intussusception.

birefringence of the wall to turn into a positive one. As little as 8 % elongation is enough to bring this about in the case of the *Avena* coleoptile (BONNER, 1935; un-

fortunately the photo-elastic aspect of birefringence was left out of account in this important publication). Upon release of the stretched system, the bars of the frame tend to revert to their original position. The photo-elastic effect disappears and the initial birefringence is seen again, though not as a rule as distinctly as heretofore since, the junctions not being ideally elastic, some of the effected deformation of the frame persists as plastic extenstion.

Considerably greater extension is involved so soon as optically positive reaction is imparted to the cell wall by *plastic* — which is to say lasting — *deformation*, this overcoming the elastic forces. From elliptical rhombs the meshes of the frame are gradually transformed to circles. In this state all the optical effects of the individual bars neutralize each other, when the membrane has a foliate texture (see page 67) and therefore appears to be isotropic. Upon further extension the meshes again become elliptical, now, however, no longer running perpendicular, but parallel to the cell axis, with the result, that the birefringence is positive. It will be seen from Fig. 129b that artificial stretching of this kind narrows the elongated area, since it is not accompanied by surface growth. Hence with this mode of extension the cylindrical cells achieve their elongation at the expense of a corresponding loss in diameter.

As no such forfeiture occurs in nature, an entirely different mechanism must underlie the elongation of the cell wall, keeping, as optics require, the cellulose filaments in their common position and allowing surface growth. These requirements are met if the junction points are loosened and the lattice rods can separate by parallel displacement (Fig. 129c). The area then becomes larger, because spaces are formed which are free from cellulose. The loosened structure may become more compact as fresh cellulose chains are woven in between the separated strands. It is this which constitutes intussusception. It is also responsible for the fact that, despite the greater surface increase, the cell wall does not as a rule become measurably thinner during linear growth, as might be expected without commensurate increase in membranous substance. OVERBECK, it is true, noticed some shrinking of collenchymatical corner thickening in the sporogonium foot of *Pellia*, but this was due, not to any passive stretching of the membrane, but to active disintegration and renewed distribution of this wall substance. Only with the co-operation of a living, formative substance can any such regrouping take place, so that the growing meristematic membrane appears to be living. Although THIMANN and BONNER detected no phosphatides and only 1 % of protein in the growing cell walls of *Avena* coleoptiles, there must be some active relationship between the living plasm and the enormously developed intermicellar system of the primary cell membrane.

Altogether, vegetable elongation growth is a far more complicated process (FREY-WYSSLING, 1945a) than has hitherto been assumed, cell elongation having been considered mainly as turgor extension. Despite the very rapid lengthening of the cell (0.3 to 7 μ in one minute), considerable growth of plasm is demonstrable by the marked increase in the amount of coagulable nitrogen (BLANK and FREY-WYSSLING, 1941, 1944). There is a simultaneous increase of all the cell wall substances as well (WIRTH, 1946). In the elongating cells of maize coleoptile, the cellulose content of the cell walls is tripled during the extension from 26 mm to 60 mm coleoptile length.

Detachment of junctions. The view that the junctions in the cellulose frame loosen up during cell elongation is exemplified by the investigations carried out by HEYN (1931), SÖDING (1931), ZOLLIKOFER (1935) and others into the plasticity of the cell wall. The primary valence chain bundles of the membrane frame lose their micellar

cohesion to some extent and may slip past each other. The wall is softened hormonally by added growth substances which loosen the junctions indirectly through the protoplasm. After extension, the junctions "harden" again and the membrane solifidifies.

It would be premature now to wish to analyse the whole chain of reactions from the arrival of the minute amounts of auxin in the cell, which would never

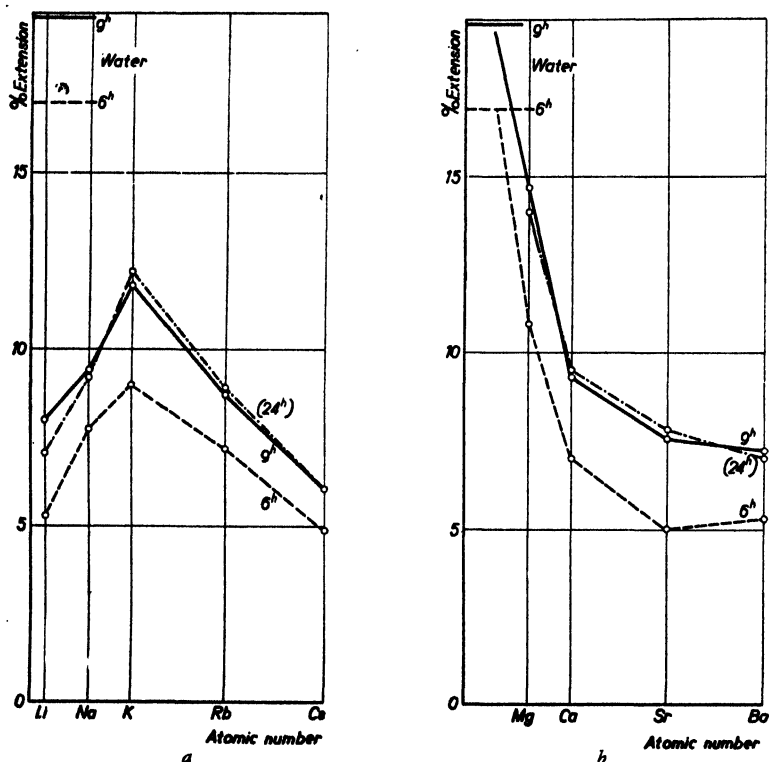



Fig. 130. Impeded endogenous effect of heteroauxin through neutral salts in cut Avena coleoptiles (after WUHRMANN 1937). *a*) Alkali chlorides; *b*) Alkaline-earth chlorides. Abscissa: Elements arranged according to their atomic number. Ordinate: % extension of portions of coleoptiles, - - - after 6 h., ——— after 9 h., -.-.- after 24 h. Top left: the elongation attained without addition of salt.

suffice to dissolve the junction points directly, up to the elongation of the walls through the plasm. It should nevertheless be possible to clarify at any rate the intrinsic nature of the junction linkages.

If a cellulose lattice really does impart solidity to the wall, the bridges must surely be of the heteropolar cohesive or subsidiary valence bond type between OH groups, hence —— OH . . . OH ——. As explained on page 99, the cohesion of these junction points depends upon the degree of hydration of the OH groups. The more they are surrounded by orientated water dipoles, the weaker will their cohesion be. Thus hydration should loosen such junction points, whereas dehydration should strengthen them. It is possible to change the state of hydration of the membrane by the addition of differently hydrated ions. Thus, if we wish to find out about the detachment of junction points, we should examine elongating cells in the presence

of various salts. K. WUHRMANN (1937) undertook this and found that the cell elongation in *Avena* coleoptiles fostered by natural auxin, or by artificially supplied heteroauxin, is hindered when salts are added. Compared with distilled water, in which more than 15 % elongation takes place, all alkali and alkaline-earth chlorides in a 0.01 n. solution very considerably check the longitudinal growth of the coleoptiles. Alkaline earths impede elongation in the following order: Mg < Ca < Sr < Ba increasingly, i.e., in inverse ratio to the hydration of the ions (Fig. 130b). With the alkali ions, on the other hand, there is an optimum curve showing Li > Na > K < Rb < Cs (Fig. 130a). In this case the plasm is damaged by the easily permeating Li and Na ions, with the result that the coleoptiles do not grow as much as the plasticity of the cell wall would give one to expect. In spite of this complication caused by the great toxicity of the highly hydrated ions — a complication in which also magnesium is involved when action is more prolonged – it was proved beyond question that the shrinkage of the membranes caused by the state of hydration of the ions used, acts as a restrictive factor in cell elongation. This conclusively proves that the enhanced plasticity of the meristematic cell walls due to hydration and their hardening caused by dehydration are brought about by secondary valence bridges.

Fig. 131. Osmotic conditions during the elongation growth of single cells in wheat root (compounded from various illustrations in BURSTRÖM 1942). Abscissa: Time (time unit is duration of mitosis in the tip of the root). Ordinates: *a*) Length of cell in μ; *b*) Turgor extension in μ; *c*) Turgor pressure in at; *d*) Osmotic material per cell in (10 μ)³ at. (After FREY-WYSSLING 1945a).

Further research, however, will have to show how this reversal of hydration is brought about by the plasm in natural growth, since, with the ions used, only some degree of solidification could be attained and none of that extensive loosening effected by the growth substances in the presence of distilled water. It might be thought that H ions are responsible for hydration of the junction points, as was certainly the case in the so-called acid growth in STRUGGER's experiments (1934); but in natural elongation, neither the auxin nor the plasm causes any so striking an acidification of the cell wall (BLANK and DEUEL, 1943).

Forces of growth. Once loosening of the junction points has rendered the micellar frame plastic, it requires a force to accomplish the work of growth. Evidently the available turgor operates, in the sense that, so soon as the wall yields and its tension is lowered, the turgor pressure, which is equal and opposite in sign to the wall pressure, will try to reestablish the original wall tension by expanding the membrane. The increased volume of the cell necessarily lowers its osmotic value owing to water absorption; consequently both turgor and suction pressure of the cell can be maintained at the original level only by a suitable osmotic regulation, which is the assumption for all turgor growth.

BURSTRÖM (1942) has carefully observed these conditions of cell elongation in wheat root. It is clear from Fig. 131 that the turgor pressure temporarily decreases during the lengthening of the cell. To raise it to its initial level, osmotic material has to be brought into the cell. Since energy is required to transport material (ARISZ, 1943), there must be considerable respiration during the elongation of the cell (BONNER, 1936b). This proceeds, therefore, not only by means of osmotically accumu-

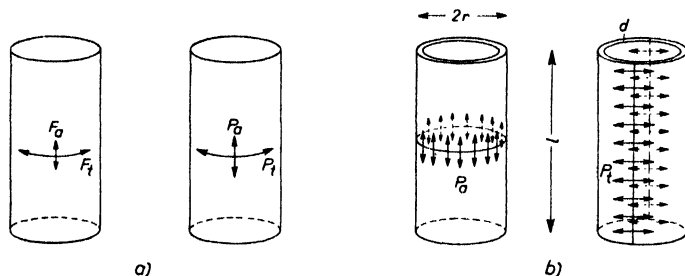



Fig. 132. Wall tension in cylindrical cells. *a*) Anisotropy of the strength F and of the wall tension p axially (index a) and tangentially (index t); *b*) derivation of longitudinal (p_a) and lateral tension (p_t). l = length, r = radius of the cell, d = thickness of the cell wall.

lated potential energy, but chemical respiratory energy is needed as well. Turgor extension is at its greatest at the moment when turgor pressure is at its lowest, from which it follows that the wall then has its maximum elasticity. Afterwards elasticity is obviously reduced by intussusception. Thus wall expansion and intercalation of substance occur simultaneously as the cell lengthens.

It is curious that, despite the turgor, the stretchable, short-cylindered cells show no tendency to become spherical during the extension. This is due to the submicroscopic tubular texture of the cells, which resists any such tendency. The strands of the cellulose frame, which encircle the cell horizontally to obliquely, have considerable tensile strength which is comparable to that of bast fibres and is due to primary valence bonds. In the axial direction, however, these rings are held together only by junction points consisting of much weaker secondary valencies. Consequently, a cylindrical cell of tubular texture has less strength axially than tangentially (Fig. 132a). It is therefore not difficult to understand that the submicroscopic, annular cellulose strands are drawn apart by the turgor elongation in an axial direction.

The turgor tension in the cell wall likewise differs according to the direction, and in the same sense as the strength of the wall. As the equation (CASTLE, 1937b) wall tension p · cross section of wall = turgor pressure T · liquid cross section applies, we have

$$p_a \cdot (2\pi r d) = T \cdot \pi r^2$$
$$p_t \cdot (2 l d) = T \cdot 2 r l$$

for the axial (p_a) and tangential (p_t) wall tension, where d is the thickness of the cell wall, r the radius and l the length of the cylindrical cells (Fig. 132b). The resultant ratio of p_t to p_a is 2 : 1, i.e., the tangential wall tension is double the axial wall tension. Although the lateral stretch in the extending cell is twice the longitudinal stretch, it grows in length only. This is possible if the $F_t : F_a$ strength ratio is above 2, as there is every reason to think it will be, since primary valence bonds are chiefly responsible for F_t, whereas merely cohesive forces, which are ten times smaller, determine F_a (see Tables III and IV). This means to say that cell expansion, instead of going along with lateral tension, follows the weaker longitudinal pull.

CASTLE (1937b), however, states that the great tangential tension prevents disorientation of the cellulose strands, thus preserving the tubular texture during extension. The prevailing conditions during the exceedingly rapid elongating growth (more than 2 mm per min) of rye stamens will serve to exemplify this view (FREY-WYSSLING and SCHOCH-BODMER, 1938). Although, according to Table XXII, the filaments increase in length by roughly 400 %, their negative birefringence persists during the entire process of extension (tests applied to stamens in different stages of elongation). The predominantly lateral orientation of the micellar frame must therefore be conceived as maintained by the tangential pull despite the tremendous turgor extension. If the extended filaments are plasmolyzed with cane sugar, their width and retardation of light differ but little, from which it is to be inferred that the birefringence observed is not brought about photo-elastically. If, however, the filaments are relieved from tension by alcohol, considerable lateral shrinkage results, which obviously re-orientates the micellar frame, as the birefringence then changes and the retardation becomes strongly positive.

Meanwhile, it remains doubtful whether mechanistic considerations of this kind are capable of entirely solving the problem. CORRENS, who noted the predominance of lateral tension in cylindrical cells as far back as 1893, came to the conclusion that "the existing pulling effects" in the micellar texture of laminated membranes of filiform algae "cannot be responsible for their orientation" (1893, p. 284), since laterally and longitudinally orientated systems occur alternately. The theory of orientation discussed, therefore, should from the start be limited to the microscopically non-laminated primary cell walls.

TABLE XXII

ELONGATING GROWTH OF THE STAMENS OF RYE. (AFTER FREY-WYSSLING and SCHOCH-BODMER 1938)

Pasted Filaments	Before extension	After extension	Plasmolysed with cane sugar		Fixed with alcohol	
				% shortened		% shortened
Length in mm . . .	3.1	15.0	10.9	(—27.3)	13.3	(—11.2)
Width in μ	112	103	112	—	62	(—39.8)
Retardation $\gamma\lambda$ in Å	—1100	—70	—92		+275	

The secondary cell wall. According to VAN ITERSON (1927), a different principle underlies the submicroscopical structure of the secondary cell wall, viz., the direction of flow of the protoplasm depositing the laminae of apposition. Currents of plasm can, in fact, be observed to circulate, depositing rings or bars during vascular formation. VAN ITERSON (1937) furthermore tries to explain the direction of flow causally. It is, he says, principally axial in the staminal hairs of *Tradescantia*, for example, since, owing to the tubular texture of the cellulose membrane, the cells tend to elongate; true the outer cuticular layer with fibrous texture impedes extension, but there is pronounced elongation the moment the cuticularized outer layer of withered flowers bursts. On the basis of these observations it was inferred that, owing to the tubular texture of the primary wall of embryonic fibres, the plasm likewise circulates in an axial direction and the nascence of the fibrous texture of the secondary wall could be explained as being causally mechanistic. VAN ITERSON now goes so far as to suggest as an explanation for the crosswise layers of the *Valonia* cell wall (brought into prominence by X-ray investigation) that the protoplasm is forced to change its

direction of flow by about 90° after the deposition of every layer; for the tendency of the cell to expand is always perpendicular to the direction of the fibrillae of the newly formed layer, for which reason the flow of plasm is supposed to be passively directed cross-wise over the youngest lamella.

With all due admiration for VAN ITERSON's sagacious reasoning, and conceding a certain contributory formative rôle to the forces he has discovered, it can scarcely

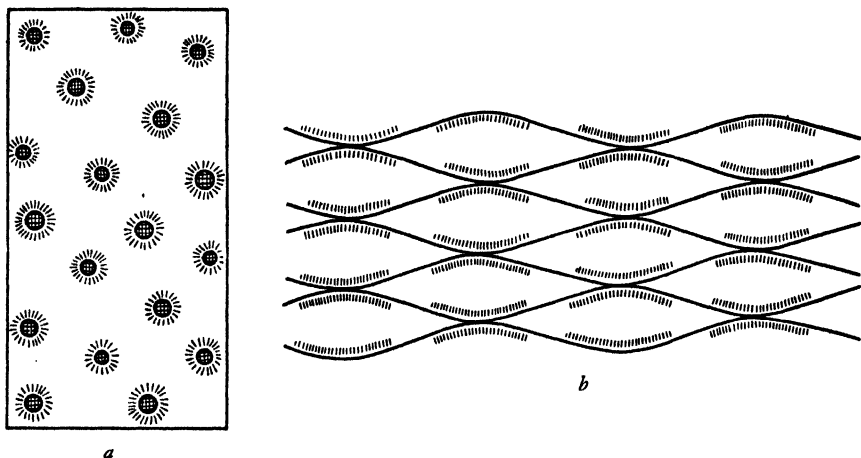
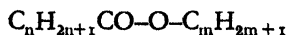



Fig. 133. Diagram of intercalation of wax in meristematic cell walls of tubular texture. *a*) Radial section; *b*) Tangential and cross sections.

be said that mechanistic theories of this kind are at present adequate to resolve the mysteries of morphogenesis. For there are several facts of observation which do not come within the compass of causation. For instance, out of similar cells near the cambium, to the primary wall of which tubular texture is ascribed, are differentiated on the one hand fibres with spiral texture, which may have been generated as suggested above, but on the other hand, vessels with tubular texture. This might be due to the fact that the fibres have top growth, whereas the vascular members have not; it would then, however, have to be explained why in one case the extensibility of the primary wall of tubular texture is utilized, while the cylindrical cells of the vascular members follow the unconventional course of growing in girth instead of in length. It is difficult to avoid assuming the existence of internal formative forces when the purposiveness inherent in each individual cell development becomes apparent again and again.

Intercalation of wax. The discovery by X-ray of the intercalation of wax has enriched our knowledge of the structure of the primary cell walls. In young cotton hairs, *Avena* coleoptiles without epidermis and many meristematic tissues, HESS and co-workers (1936) found X-ray interferences corresponding to periods of 60 and 83 Å. By extraction these substances were isolated and identified as vegetable waxes (GUNDERMANN, WERGIN, and HESS, 1937). They are comparatively short chains of the

$$C_nH_{2n+1}CO\text{–}O\text{–}C_mH_{2m+1}$$

type, n and m amounting to about 24 of 32, as established for other vegetable waxes by CHIBNALL, PIPER, and co-workers (1934).

As these waxes produce far clearer interferences than cellulose, of which often

only the fibre period appears, they must be assumed to be better crystallized than the cellulose chain molecules. The possibility therefore exists that waxes of this kind are in part the source of the birefringence of the primary cell walls. Pursuing this problem as presented by the meristematic cell walls of *Avena* coleoptile, K. and M. WUHRMANN-MEYER (1939) established that the birefringence is affected by the fatty wax component susceptible of extraction by pyridine. Though this effect is, admittedly, lacking in the radial sections through the cells, it appears in the tangential and cross-sections. From this it may be inferred that the rod-shaped wax molecules are orientated at right angles to the micellar strands of the tubular texture; then there is isotropy on the radial section, whereas on the tangential and cross-sections we have a birefringence which is the reverse in character of that of cellulose, as will be clear from Fig. 133.

The waxes being extremely hydrophobic and the cellulose chains very hydrophilic, there can be no direct contact, between these two membranous substances, so that an intermediate, polar substance is interposed (FREY-WYSSLING, 1937d). Possible molecules with hydrophilic and hydrophobic end groups are phosphatides (HANSTEEN-CRANNER, 1926) or proteins. Seeing that THIMANN and BONNER (1933) found no phosphatides in the membranes of *Avena* coleoptile and that no proteins could be detected microchemically in the cell wall, the question arises as to whether the wax alcohols and fatty acids in the primary walls occur in the unesterified state, in which case their hydrophilic pole would be connected with the cellulose threads. It will be evident from Fig. 133 why the primary cell walls can be stained with fatty acid, whereas the individual cellulose strands seem to be "masked". Physiologically this intercalation of wax results in the impaired permeability of the wall to water, ions and lipophobic molecules, as these substances are admitted, not through the entire meshes of the intermicellar spaces but only through the hydrophilic regions in the vicinity of the cellulose strands.

b. *Cutinized Cell Walls (Cutin)*

Microchemistry and optics of cutinized epidermises. The morphology of the thick cuticular layers of the leaf epidermises of xerophytes (FRITZ, 1935, 1937) is particularly interesting, in that, although optically often appearing to be homogeneous, they contain at least four different membranous substances, the submicroscopic arrangement of which is known. Our starting point will be the optics, investigated by AMBRONN (1888), of the cuticular layers which, in the polarizing microscope, behave in a reverse sense to the cellulose layers lying beneath them. The cellulose component appears optically positive with reference to the tangential direction of the cell wall, while on the contrary the cuticular layer is optically negative (Fig. 134a). Externally, the epidermis is bounded by the optically isotropic cuticle and between the cellulosic and cuticular layers is interposed a fairly wide, likewise isotropic layer of pectins (ANDERSON, 1928). AMBRONN had already suspected that the optically negative reaction of the cuticular layers was caused by intercalated waxes, but this property was later attributed to the cutin. MADELEINE MEYER (1938), however, demonstrated by careful micromelting tests (Fig. 134b) that the negative birefringence derives from a fusible wax, while the residual cutin frame proves to be isotropic. In many cases, of which *Gasteria* is an example, a slightly positive birefringence, proceeding from cellulose, makes its appearance after the waxes have melted out. Hence, besides the cutin, the cuticular layer must also contain cellulose and even pectins, which can be identified by ruthenium red. The optics of the longitudinal

TABLE XXIII

CELL WALL SUBSTANCES OF THE CUTICULAR LAYERS

	Optical behaviour referred to tangential direction	Coloured by	Solubility	Disintegration by	UV Absorption
Cutin	Isotropic	Basic lipid dyes	Insoluble	NaOH saponification	Strong
Cutin waxes	Opt. negative	Lipid dyes	Pyridine	Melting above 220°C	Lacking
Cellulose	Opt. positive	Iodine-zinc chloride sol. (dichroism)	SCHWEIZER reagent	Hydrolysis	Lacking
Pectins	Isotropic	Ruthenium red	Picric acid followed by H_2O_2	Hydrolysis	Lacking

section disclose the fact that these four membranous substances (Table XXIII) are not evenly distributed over the thickness of the cuticular layer. In *Clivia*, for instance, only an inner zone — which iodine-zinc chloride solution tints dark brown — clearly contains cellulose. The waxes are in greatest evidence in the middle of the layer, so that it is there that the retardation is at its most negative (FREY, 1926b). The wax content diminishes outside and the cuticle contains no wax at all, consisting of pure cutin (Fig. 134a).

In hydrophytes the cutinization of the epidermis is confined to a thin, optically isotropic cuticle. It is probable that all cell walls that are in contact with air are

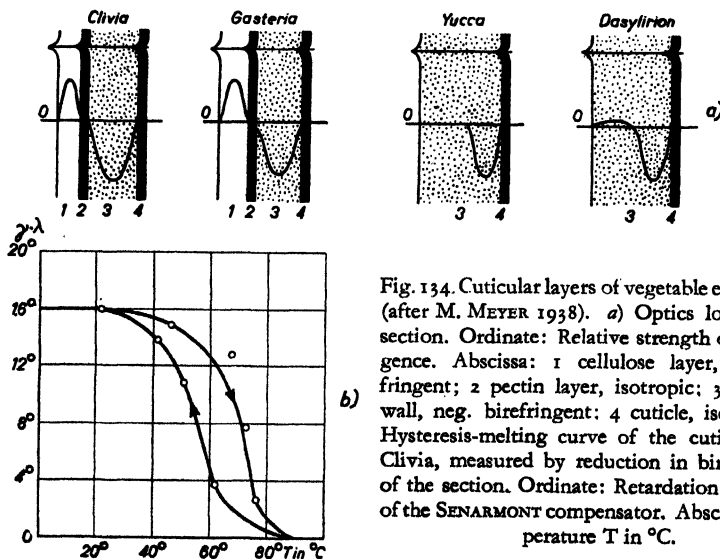



Fig. 134. Cuticular layers of vegetable epidermises (after M. MEYER 1938). *a*) Optics longitudinal section. Ordinate: Relative strength of birefringence. Abscissa: 1 cellulose layer, + birefringent; 2 pectin layer, isotropic; 3 cutinized wall, neg. birefringent; 4 cuticle, isotropic. *b*) Hysteresis-melting curve of the cutin wax of Clivia, measured by reduction in birefringence of the section. Ordinate: Retardation in degrees of the SENARMONT compensator. Abscissa: Temperature T in °C.

superficially cutinized, since ELSA HÄUSERMANN states (1944) that the cells of mesophylls, which serve to ventilate the leaf, are covered with a submicroscopical film of cutin.

Molecular structure of lipophilic cell wall substances. To understand the submicroscopical arrangement of the four cell wall substances in cuticular layers it is necessary to known the morphology of their molecules. We shall therefore have to consider briefly the chemistry of the waxes and of the very imperfectly known cutin. Unlike the enormously long cellulose chain molecules and the very long pectin chains, the waxes are, as already mentioned, short rod molecules of less than 100 Å length. In the simplest case they consist of one of the higher fatty acids, such as cerotic acid $C_{25}H_{51}COOH$, esterified with a higher alcohol, say myricyl alcohol $C_{30}H_{61}OH$. According to CHIBNALL, PIPER and their collaborators (1934), only the evennumbered chains occur, above all, between C_{24} and C_{34}. It will be seen by the linkage plan of Table XXIV that the cell wall waxes have no reactive end groups; they cannot, therefore, polymerise and are of comparatively low molecular weight. Besides the aliphatic waxes, whose overall formula, like that of fatty acid, is $C_nH_{2n}O_2$, the cutinized, and especially the suberized, membranes contain the waxes cerin and friedelin, which have a substantially lower hydrogen content. The inference therefore is that they contain aromatic rings and thus approximate the sterols, which represent the cyclic alcohols. LÜSCHER (1936) states that friedelin and cerin contain an alcoholic OH group which can be acetylated or otherwise esterified, while the second constituent O atom is masked, presumably as cyclic ether bridge. Thus friedelin and cerin are alcohols, not esters. On the other hand, they may possibly be esterified with other molecules in the membrane. Otherwise nothing is known of their constitution. On saponifying the waxes of pine needles, BOUGAULT and BOURDIER (1908) obtained ω-hydroxyfatty acids (such as hydroxylauric acid and hydroxypalmitic acid) instead of simple acids and alcohols. Molecules of this kind possess two reactive groups; thus they can together form esters and grow to high-polymeric chains, as shown in Table XXIV. Their discoverers call these waxes "estolids". Their degree of polymerisation cannot be very high, as they are still soluble and fusible.

The polymerisation plan of the high-polymeric cell wall substances cutin and suberin must be similar to that of the estolids, since their hydrolytic and decomposition products ordinarily exhibit two or more reactive groups capable of esterifying or etherifying (dicarboxylic acids, hydroxycarboxylic acids, Table XXIV). This is the distinguishing feature between the monomeric molecular residues of cutin and suberin, on the one hand, and the molecules of waxes on the other (LÜSCHER, 1936). Seeing that suberin is more readily decomposed than the cutins (ZETZSCHE, 1932), it is probable that the degree of polymerisation or of interlinking attained within it is lower than in the latter. It is presumably at its highest in sporopollenin, as this wall substance is exceedingly resistant to saponification and decay, so that the cell walls of fungus spores and the grains of pollen are preserved for thousands of years in peat deposits.

The isolated dicarboxylic acids (Table XXIV) may possibly be oxidized degradation products of higher hydroxyacids; suberic acid, $COOH \cdot (CH_2)_6 \cdot COOH$, for instance, results from the oxidative degradation of suberin. Probably not all the carboxyl groups of the carboxylic acids in the membrane are esterified, for cutin has some of the characteristics of an acid, or high-polymeric anion (pronounced negative charge BRAUNER, 1930, selective cation permeability, staining by basic dyes). Since its behaviour is optically isotropic, it must be presumed that the linkage of the carboxyl and hydroxyl groups is not that of a linear chain scheme, but reticular in all spatial directions as in lignin.

Submicroscopic structure of the cuticular layers. It now remains to build up a picture of the mutual spatial relationship between the cell wall substances in the cuticular layers. A possible clue is afforded by the optical anisotropy of the submicroscopic particles of wax. If their form and optics were known, the orientation of the intercalated wax could be inferred from the nature of the wall birefringence.

TABLE XXIV

LIPOPHILIC CELL WALL SUBSTANCES

Aliphatic Waxes:	*Wax Acids:*		*Wax Alcohols:*	
$CH_3 \cdot (CH_2)_n \cdot C(=O)—O—(CH_2)_m \cdot CH_3$	Palmitic acid	$C_{15}H_{31}COOH$	Cetyl alcohol	$C_{16}H_{33}OH$
	Stearic acid	$C_{17}H_{35}COOH$	Octadecyl alcohol	$C_{18}H_{37}OH$
	Oleic acid	$C_{17}H_{33}COOH$	Ceryl alcohol	$C_{26}H_{53}OH$
	Linoleic acid	$C_{17}H_{31}COOH$	Myricyl alcohol	$C_{30}H_{61}OH$
(CHIBNALL and PIPER, 1934;	Arachic acid	$C_{19}H_{39}COOH$	Higher alcohols up to	$C_{34}H_{69}OH$
LÜSCHER, 1936)	Cerotic acid	$C_{25}H_{51}COOH$		
	Higher fatty acids up to	$C_{33}H_{67}COOH$		

Cyclic Waxes:	
Molecular structure:	Cerin $C_{30}H_{50}O_2$
(LÜSCHER, 1936)	Friedelin $C_{46}H_{76}O_2$

Estolids:	*Hydroxyacids:*	
$—O—(CH_2)_y—C(=O)—O—(CH_2)_y—C(=O)—O—$	Sabinic acid (hydroxylauric acid)	$OH \cdot C_{11}H_{22} \cdot COOH$
(BOUGAULT and BOURDIER, 1908)	Juniperic acid (hydroxypalmitic acid)	$OH \cdot C_{15}H_{30} \cdot COOH$

Suberin, Cutin and Sporopollenin:	*Decomposition Products of Suberin:*	
Molecular structure: Spatial network through ester and ether bridges	Suberic acid	$COOH \cdot (CH_2)_6 \cdot COOH$
(ZETZSCHE, 1932; LÜSCHER, 1936)	Phloionolic acid	$C_{17}H_{32}(OH)_3 \cdot COOH$
Suberin ↓ Saponification	Phloionic acid	$COOH \cdot C_{16}H_{30}(OH)_2 \cdot COOH$
Cutin ↓ becomes more and	Phellonic acid	$C_{21}H_{42}(OH) \cdot COOH$
Sporopollenin ↓ more difficult	Eicosanedicarboxylic acid	$COOH \cdot (CH_2)_{20} \cdot COOH$

The wax molecules are rod-shaped and therefore, when spread on a slide, might be expected to be orientated and reveal something as to their intrinsic birefringence. Many waxes, like paraffin, fats, phosphatides and other lipids, produce what is known as a "negative streak" (Fig. 135b), which might incline one to conclude that the wax molecules are optically negative with reference to their longitudinal axis. Such a conclusion is, however, inadmissible, since short-rodded molecules have a tendency to crystallize as thin platelets or lamellae (Fig. 135a) and, when spread out, these submicroscopic, often plastic, crystal lamellae are orientated. Longitudinally, they fall in with the direction of the stroke and the molecules then run perpendicular to the streak. In this way the streaks of paraffin and beeswax are negative, but the molecules themselves are optically positive[1]. By analogy it might therefore be sup-

[1] On p. 65 it is explained that double refraction cannot be attributed to a single molecule. So if we speak here of optically positive molecules, this means that the sign of the double refraction of a large number of molecules, parallelized by flow or crystallization, is positive.

posed that the molecules of the vegetable waxes which yield a negative streak are positive; but there are some waxes with a positive streak, as I found with estolids from pine needles (Fig. 135c). The streak test, therefore, tells us nothing definitite and another method has to be resorted to, which consists in dissolving the waxes, in order that their molecules may be rendered independent of each other, and then testing their intrinsic birefringence in the flow gradient.

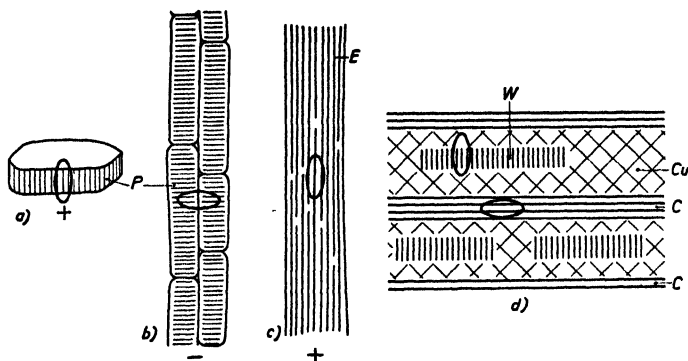



Fig. 135. Submicroscopic textures from optically positive lipid chains. P = paraffin molecules, E = estolid molecules, W = wax molecules, C = cellulose chains, Cu = interlinked cutin chains. *a*) Paraffin lamellae optically positive; *b*) optically negative streak of paraffin; *c*) optically positive streak of pine-needle wax; *d*) intercalation of cutin wax in the epidermis.

AMBRONN and FREY pointed out in "Polarisationsmikroskop" (1926, p. 167) that the only certain way of establishing the intrinsic birefringence of disperse particles is by using a rotary drum in accordance with KUNDT's system. SIGNER (1930, 1933) built a flow-birefringence apparatus of the greatest precision, in which, in spite of their Brownian movement, comparatively short rod-molecules can be orientated. It was with the aid of this apparatus that WEBER (1942) determined the optical nature of wax molecules. The experimental evidence points to optically positive rod-molecules. *Thus the molecules of the membrane waxes, like those of paraffin, fats and other lipids, are optically positive rodlets.*

Since the waxes, referred to the tangents of the cuticular layers, produce negative birefringence, their molecules must stand perpendicular to the surface of the membrane. So perfect is the orientation of the rod-molecules, that the outside layer of the epidermis of *Clivia*, seen from above after the removal of the cellulose layer underneath it, appears optically isotropic. Hence the cuticular layer possesses a radial optical axis and its birefringence should therefore by rights be described as positively uniaxial. The introduction, however, of this reference axis for the purpose of designating the birefringent phenomena in the cuticular layer of the epidermis is not without its drawbacks, as it does not represent any preference direction for the cellulose frame.

After extraction of the wax, textural birefringence was exhibited (textural birefringence curves in M. MEYER, 1938), this, referred to the optical axis of the cuticular layer, being negative. This means that we have to do with lamellar birefringence; hence the wall layer consists of submicroscopic lamellae, in the texture of which, judging by all previous experience, the cellulose of the cutin layer must be involved. The optical analysis therefore suggests the presence of submicroscopic cellulose

lamellae with exceedingly thin platelets of wax interposed, the wax molecules being orientated perpendicular to the cellulose chains (see Fig. 135d).

Now, in the presence of the water, present not only in cellulosic, but also in cutinized cell walls, the hydrophobic wax molecules cannot come into contact with the hydrophilic cellulose chains. Thus there must be some intermediate polar substance, and that is the cutin. This wall material contains both hydrophilic (–OH, –COOH) and hydrophobic ($-CH_3$) groups and it may be assumed that the former incline more towards the cellulose, whereas the latter tend more towards the wax. We then have a scheme such as that represented in Fig. 135d.

It can be seen in this model how the cell wall substances in the cuticular layers are placed one to another: hydrophilic lamellae consisting of cellulose and probably also of pectins, layers of wax molecules in radial arrangement and, in between them, amorphous cutin in random orientation. Apart from the interposition of the wax, the morphological conditions are similar to those in lignification, where amorphous lignin is intercalated between cellulose rodlets or lamellae. In both cases the cellulose is masked by the incrustation, certain groups of molecules probably sharing secondary, or even primary, valence bonds at the places of contact between the two different wall substances. For example, it was only with difficulty that the cellulose could be dissolved out of wood with SCHWEIZER's reagent, and hitherto could not be eliminated at all in this way from the cutin layers. It is easier to saponify the cutin, or the suberin (KARRER and PEYER, 1923, M. MEYER, 1938) and to liberate the cellulose.

The scheme shows the relative positions of the four cell wall substances, not their quantitative proportions, these being very variable. Small to large ramounts of the carbohydrate wall substances, cellulose and pectins can always be identified in the inner regions of the cuticular layer; they are, indeed, often quite prominent. More to the outside, it is the waxes which are in greater prominence, with marked and sometimes complete decline of cellulose and pectins. The outer layers probably consist of cutin and wax only. This is noteworthy as compared with lignin deposition, since cutin can obviously occur as an independent wall substance, whereas lignin is always found in company with cellulose. Finally, there are no waxes in the isotropic cuticle[1], which, therefore, comprises only a thin pellicle of amorphous cutin.

It would be interesting to discover the still quite unknown history of the development of this complicated submicroscopic system originating in a region remote from the plasm. MARTENS (1934) states that the cuticle is secreted in the fluid state and then coagulates in the air. This may also safely be said to apply to the cuticular layers. The cutinic acids would then be dissolved in a low molecular state, immigrate into the wall and there polymerize. It is less difficult to understand the deposition of the low-molecular waxes, though even in this case it is necessary to assume that there is some special solvent, or that unesterified wax acids and alcohols migrate. This process is similar in nature to the excretion of waxes through the epidermis, where they form a granular, rod-shaped or scaly coating (WEBER, 1942).

All components of the wall in the full-grown cuticular layer have their peculiarized physiological function. By reason of its hydrophobic nature, the primary duty of the wax is to make these layers *watertight*. The cutin has a similar purpose, though in a less extreme degree, since its hydrophilic groups make it less hydrophobic and, therefore, it has a slight tendency to swell. As the cutin layer strongly absorbs ultraviolet light (FREY, 1926b) and retains this property even after the waxes have been

[1] PRIESTLEY (1943).

extracted, it impedes any intensive *ultraviolet radiation* of the mesophyll of xerophytes. As aliphatic compounds in general do not absorb ultraviolet light, there must be some unknown cyclic compound (cyclic waxes) in the cuticular layer. The hydrophilic quality of the lamellae of cellulose and pectins is responsible for the *cuticular transpiration* (GÄUMANN and JAAG, 1936) of the leaves, which occurs not only in hydrophytes, but also in xerophytic evergreens. The loss of water is a sign that the submicroscopic wax lamellae are not continuous, somewhat like the microscopic wax layers on the leaves of wax palms, but that the hydrophilic (cellulose) and semi-hydrophilic (cutin) regions cohere and thus offer the water an outlet.

c. *The Chitin Frame (Chitin)*

Chitin is a nitrogenous frame substance, primarily characteristic of the animal branch of *Arthropoda* (*Crustacea*, insects). It also forms the membranous frame of *fungi* (HARDER, 1937), however, and the behaviour of vegetable and animal chitin is identical, as has been proved for the sporangiophores of *Phycomyces* chemically, optically and by X-ray (DIEHL and VAN ITERSON, 1935; VAN ITERSON and MEYER, 1936). In the same way as the cellulose characteristic of autotrophic plants may be built by both bacteria (*Bacterium xylinum*) and by the animal branch of the *Tunicata*, *fungi* are, inversely, able to synthesize an animal frame substance. One cannot go very far wrong by assuming that this similitude is connected with the heterotrophic life of fungi, which, like animals, have so much nitrogen to draw upon that some of it is deposited in the cell walls and is there immobilized. As there is, on the contrary, only a minimum of nitrogen in autotrophic plants, it cannot contribute to the formation of the frame substances; otherwise chitin, which is more resistant than cellulose in many respects, would surely also occur elsewhere in the vegetable kingdom. Morphologically, the two frame substances are very similar in behaviour, as will be shown in what follows, the micellar frame of each being composed of very long chain molecules.

Molecular structure of chitin. The structural unit of chitin is glucosamine, i.e., a pyranose ring in which an OH group has been substituted by an NH_2 group (Fig. 136a). It is not known whether the position of this amino group at the 2ndC atom corresponds to that of the OH group of the glucose or of the mannose ring (ITSCHNER, 1935). An acetyl residue is linked with the NH_2 group; thus, contrary to cellu-

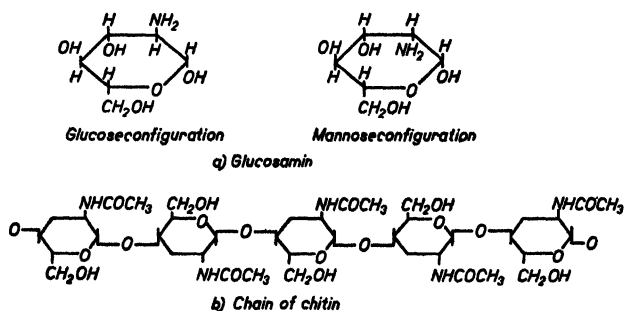



Fig. 136. Molecular structure of chitin.

lose, there are here two side chains, viz., an $OHCH_2$-group and a CH_3CO-group.

The acetylglucosamine molecules are linked glucosidically and form long chain

molecules, each member of which is, according to MEYER and MARK (1930), twisted with respect to its preceding and succeeding neighbour by 180° (Fig. 136b). X-ray photographs of the sinews of spiny lobster and of the sporangiophores of *Phycomyces* show that the crystallographic elementary cell is rhombic, its dimensions are 9.4 : 10.46 : 19.25 Å and it contains eight acetylglucosamine residues, viz., two to every four main valence chains, which traverse the crystal lattice (MEYER and PANKOW,

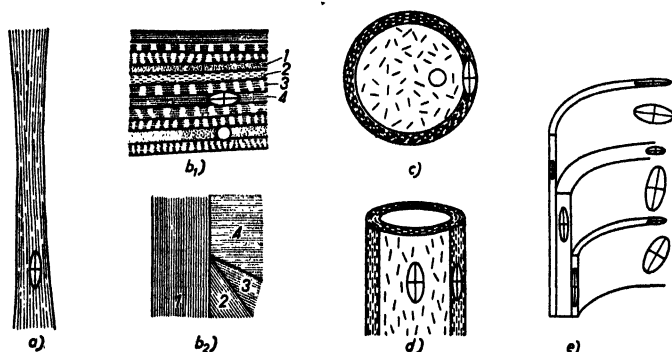



Fig. 137. Types of fine-texture with chitin as the frame substance. *a*) Crab sinew: fibrous texture. *b*) Interior of lobster shell: submicroscopic lamellar texture, with direction of fibrillae changing from lamella to lamella (1, 2, 3, 4, etc.); b_1) cross section, b_2) plan. *c*) Eggshell of Ascaris: foliate texture (SCHMIDT 1936b). *d*) Conidiophores of *Aspergillus*: fibroid texture (FREY 1927*a*). *e*) Conidiophores of *Phycomyces* (OORT and ROELOFSEN 1932): spiral texture.

1935). The fibre period 10.46 Å is important, because it corresponds to the length of two pyranose rings and is identical to that of cellulose. This warrants the belief that the glucosane rings, like the glucose residues of cellulose, are linked together by β-glucosidic 1–4 bonds (see Fig. 136b).

Submicroscopic texture of the chitin frame. Microscopically, the chitin sheath of the *Arthropoda* and the membranes of *fungi* show lamellation and fibrillation, as is known to be the case in the cell walls of cellulose. By analogy, therefore, it may be assumed that fibrillation is realized in the submicroscopic region, so that the micellar frame is built up of long, submicroscopic rodlets. The intermicellar spaces in crustacea are filled partly with mineral substances, especially with calcium carbonate, while the membranes of fungi are incrustated with substances rather of a carbohydrate or pectinous nature (which can be extracted by boiling for several hours with a ten percent solution of caustic potash).

As with cellulose, the orientation of the rods of the frame is demonstrable by optical means, since the larger axis of the index ellipse of sections immersed in water or glycerol runs parallel to the submicroscopic chitin rodlets. This method reveals the same potential orientation as that actualized in cellulosic cell walls (Fig. 137).

Chitinous tendons of crabs, lobsters, beetles, etc. are of an unmistakable fibrous texture. Of all chitinous objects, therefore, they produce the most richly pointed X-ray photographs and are thus the most informative as to the lattice structure of chitin. Optically, the fibrous texture is disclosed by the fact that the refractive power is considerably more pronounced parallel to the axis of the tendon than perpendicular to it, while something like isotropy prevails in the cross sections of the tendon. This fibrous texture is to be inferred, not only from the birefringence, but also from

the anisotropy of the absorption of light. Iodine-zinc chloride solution and Congo red stain decalcified and cleaned chitinous tendons, as they do bast fibres, dichroitically; that is to say the direction of the stronger light absorption coincides, as in cellulose, with the fibre axis. The similarity in the dichroitic coloration of chitin and cellulose is interesting in that it tends to show that the dichroism of these colour reactions characterizes not so much a certain chemical compound as its micellar structure with orientated inner surfaces.

The egg-shell of *Ascaris* provided SCHMIDT (1936b) with an object in which the submicroscopic chitin rodlets scatter, thus forming a wall of foliate texture. The plan of the eggs shows them to be isotropic, but the optical cross section through the wall exhibits a negative spherite cross. This optical behaviour is produced by an arrangement of the submicroscopic ordered lattice regions as represented in Fig. 137c.

The sporangiophores of *Aspergillus niger* must, from their optics, be presumed to have a fibroid texture with scattering (Fig. 137d; FREY, 1927a). We do not yet know, however, whether this membrane be not stratiform like *Phycomyces*; for in that fungus, with particularly large sporangiophores several centimetres in length, OORT and ROELOFSEN (1932) found an outer primary skin of tubular texture, under which there is a secondary thickened layer of fibrous texture and exhibiting slight scattering; it is by reason of its predominant bulk that only this appears on the X-ray photograph. It is assumed that at the core there is another, very thin layer of steep spiral texture (Fig. 137e).

Spiral growth. As the end of the sporangiophore in the zone of growth has only the primary skin, its optics is not disturbed by secondary layers. It is only beneath the zone of elongation that the secondary wall is deposited. The primary wall is conspicuously of spiral growth (OORT, 1931; CASTLE, 1937a, 1942), a fact verified by placing a mark above the zone of growth which was found not only to travel upwards, but at the same time to rotate around the axis of the sporangiophore (Fig. 138). There is nothing in the submicroscopical texture of the primary wall which might account for this behaviour. OORT and ROELOFSEN state that the isolated wall is flabby and flexible and, as it tears impartially in all directions, is not of fibrillar texture. It is therefore to be presumed that the chitin lattice regions are very much scattered. If the interior pressure in the zone of growth is artificially enhanced, the membrane bursts through a very steep spiral longitudinal tear, which may be attributed to the anisotropic states of tension in all tubular walls described on page 180. Artificial extension of the zone of growth is accompanied by a rotation which, after relaxation, recovers. Thus the optics point to tubular texture with scattering, while the mechanical properties require a spiral texture which cannot at present, however, be confirmed optically, though PRESTON (1936) assumes it to be so. CASTLE (1936b) tried to imitate spiral growth in a model but, instead of showing tubular texture with scattering, it displays a fibrous texture with converging

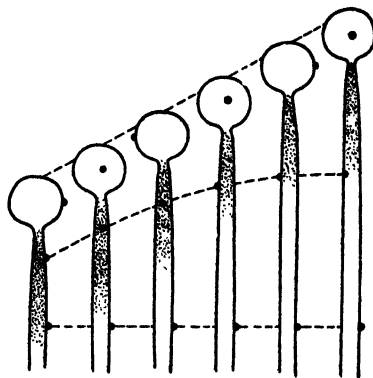



Fig. 138. Spiral growth of *Phycomyces* (according to CASTLE 1937*a*). Zone of growth dotted line; • marks to trace rotation.

rods lying in radial planes which, judging by the optics, certainly is not realized in the primary wall. The simplest assumption is that intussusceptive growth in the zone of extension travels in a circle (CASTLE, 1937a); this must be so, since the slender conical shape of the zone of growth could hardly be maintained if the surfacc grew simultaneously on all sides. In line with this is the fact observed by OORT and ROELOFSEN, viz., that in *Phycomyces Blakesleeanus* var. *piloboides* with a characteristically inflated sporangiophore, the sporangium does *not rotate*.

Spiral growth is of general interest, because it occurs not only in cytological objects, but also in organs (e.g., tendrils) or entire organisms (e.g., snails and slugs). VAN ITERSON (1943) therefore made a model for this form of growth and, contrary to CASTLE's (1936b), made it representative of cylindrical objects. In this model axially parallel elements of the cylinder jacket lengthen, while the distance between the two basal surfaces of the cylinder remains the same; under which conditions the textural elements are forced out of their axially parallel position into an oblique, spiral arrangement. Let us imagine a cylindrical cell in which the primary membrane, with its tubular texture, has already been deposited, against which the secondary wall, consisting of axially parallel micellar strands with a tendency to grow longitudinally, is deposited, and we have the conditions depicted by VAN ITERSON. Some mechanism of the kind is certainly conceivable, seeing that the secondary wall begins to be formed before the elongation growth of vegetable cells is completed (WUHRMANN–MEYER, 1939). It is PRESTON's view (1934, 1938) that the primary skin possesses a spiral texture. This, if true, would weaken VAN ITERSON's arguments, of course, but it is unlikely that the primary wall has a uniformly parallel texture, since oblique extinction, fibrillar cleavage, etc., do not become demonstrable before incipient formation of the secondary wall. To my mind a more cogent objection to the mechanistic conception of spiral texture formation is the fact that the direction spiralling will take — i.e., to the left or to the right — depends merely on chance, whereas many cases are known in which the helical direction is always the same. It would be well to recall the classical example of *Phycomyces*: after new investigations CASTLE (1942) was able to show that at first there is regularly a left tendency in growth, which then suddenly changes for an hour to a right-hand spiral and then back again to a left spiral. CASTLE tries to account for this by suggesting the preformation of both a left-hand and right-hand screw in the primary wall; that is to say, it would be a crossed system indistinguishable from the tubular texture.

This brings us to the interesting question of crossed lamellar systems, which are typical of the intrinsic texture of many biological objects.

Crossed lamellar systems. An instructive example of a microscopical laminated structure is afforded by the inner layer of the lobster shell. As an entity, this layer behaves like a uniaxial, optically negative composite body; i.e., seen from the surface, it is isotropic. In cross-section, on the other hand, strongly birefringent (positive with reference to the lamellation) and isotropic layers are seen to alternate. Older investigators (BIEDERMANN, 1903) thought these lamellae possessed cross-wise fibrillation at right angles. Were this true, it should be possible to cut cross-sections at 45° to the two fibrillar directions through the composite body in which all the lamellae would show the same behaviour in the polarizing microscope. This, however, is not the case, for cross-sections, in whatever direction, through the lobster shell all invariably disclose the same pattern of lamellation. SCHMIDT (1924, p. 238) therefore

assumes that the fibrillae in consecutive, very thin, parallel-fibred layers very gradually change direction, so that two layers at a certain distance from each other will contain fibrillae crossed at right angles, but those in between will contain fibrillae in any of the transitions from 0^0 to 90^0. An arrangement such as this is indicated in Fig. $137b_1$. This should be verifiable optically for, in the transition from lamella to lamella, the light retardation should drop following a sine curve from the maximum value to nil. X-ray analysis would likewise show whether all possible fibrillar directions are before us. It seems to me an important point that the hypothetical layers are submicroscopically thin for, were they of microscopical dimensions, it would mean that this is a comparable case to the spiral texture of cotton fibre; that is to say, owing to the obliquely crossed layers, the top view of the interior layer of the shell could not be isotropic, but would have to transmit some light under all azimuths.

Instead of assuming submicroscopic lamellae consisting of parallel microfibrillae superimposed in different directions of orientation (Fig. $137b_2$), it would be equally plausible to picture the microfibrillae as interweaving.

Vegetable cellulose membranes were studied (FREY–WYSSLING, 1941) with the object of discovering whether in laminated systems the individual layers are of parallel texture, or whether it is a matter of interweaving. We have examples, such as the algae *Valonia* (VAN ITERSON, 1933) and *Chaetomorpha* (NICOLAI and FREY–WYSSLING, 1938), the laminated cell walls of which can be split up into single lamellae of a few tenths of a μ in thickness; these lamellae are made up of strictly parallel fibrillae, which accounts for their striking cleavability parallel to the fibre direction. In consecutive lamellae the fibre directions cross at approximately right angles (in *Valonia* at 78°); consequently the optical anisotropy of the individual lamellae is to a large extent mutually neutralized and, transparently, the appearance is roughly that of statistically isotropic packets of layers. (Cp. PRESTON, 1947).

As opposed to these systems of membranes with uniform parallel texture of the individual lamellae, we have the fine-structure of the primary wall of cotton fibres. This thin membrane, which allows for the extraordinary growth of the cotton hair from approximately 40 μ to the length of 40 mm (which is a thousandfold), exhibits, according to ANDERSON and KERR (1938), three different systems of striations, one of which runs perpendicular to the fibre axis, the two others falling symmetrically at an angle of about 30° obliquely from the left and right. As the membrane cannot in this case be split up into three lamellae, presumably there are three different fibrillar directions in one and the same lamella. The micellar frame might conceivably be made up of regular submicroscopic hexangular meshes (compare Fig. 129a), the parallel sides of which would run in the three main directions (FREY–WYSSLING, 1941). Alternatively it may be supposed that submicroscopic fibrillae are interwoven in the three directions after the manner of a textile fabric.

The observations made by ROSIN (1946) of the tail edges of batrachian larvae would support the latter possibility. Judging by the arrangement of the pigment cells, which rest on a basal membrane of connective tissue, it would seem that the intrinsic texture of this membrane must consist of orthogonally trellised submicroscopic fibrillae of collagen. As it cannot be split up into two lamellae, the two systems of fibrillae apparently lie in the same plane. ROSIN was able to show how the orthogonal fibrillar system grows by affine enlargement of the surface, the trellising of the two fibrillar systems always remaining rectangular (Fig. 139). Intussusception

is responsible for surface enlargement, inasmuch as new submicroscopic fibrillae are embedded in parallel.

In nature, therefore, there are very probably crossed microstructural systems, the fibrillar structural elements of which interweave orthogonally or at other angles. The establishment of this fact may assist very materially in clarifying the submicroscopic texture of exceedingly thin membranes.

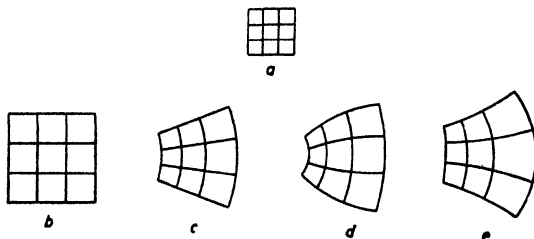



Fig. 139. Affine growth (after ROSIN 1946). *a* may grow larger progressively in proportion to *b*, or towards one side (*c*, *d*, *e*); the crossed system thereby remaining orthogonal.

Rodlet and intrinsic birefringence of the chitin frame. Chitin was the first biological object in which the interaction of textural and intrinsic birefringence — discovered by AMBRONN in artificial gels — could be demonstrated (MÖHRING, 1922). When decalcified cross-sections through lobster shell or of chitin sinews are immersed in solution of mercuric iodide of potassium of increasing refractive power, the birefringence decreases, falls to nil, changes its sign, reaches a minimum in the negative region, becomes nil a second time and then returns to positive (Fig. 140). The inference from this is that the micellar system of chitin is marked by a pronounced positive textural anisotropy, which is to say rodlet birefringence and a slightly negative intrinsic birefringence.

In his imbibition experiments CASTLE (1936a) finds reversal of the birefringence with mercuric iodide of potassium and iodobenzene in xylene, but not with other organic liquids (methylene iodide in xylene, iodobenzene in alcohol). From this he concludes that the source of the negative birefringence is not natural chitin, but chitin changed chemically by, say, mercuric iodide of potassium. This conclusion is, however, incorrect, for DIEHL and v. ITERSON (1935) found with mixtures of glycerol and quinoline, and SCHMIDT (1936b) with α-monobromo-naphthalene (mixed with xylene) negative minima of the rodlet birefringence curve. True, the curves are not identical for various imbibition mixtures (Fig. 140) and none has its minimum at $n_D = 1.525$, which is the value BAAS BECKING and CHAMBERLIN (1925) found by the immersion method for the refractive power of crab and insect chitin. What was demonstrated in cellulose (FREY-WYSSLING, 1936b) probably applies here, viz., that the difference in the adsorptive power of the micellar frame with respect to the components of the imbibition liquid is responsible for the displacement of the curves. From the data now available, therefore, it may confidently

Fig. 140. Rodlet birefringence curve of chitin sinews (after DIEHL and v. ITERSON 1935). *A* with quinoline-glycerol, *B* with mercuric iodide of potassium. Abscissa: Refractive index n_2 of the imbibition liquid. Ordinate: Birefringence n_a-n_o.

be asserted that the submicroscopic chitin rodlets have a negative intrinsic birefringence.

Cellulose likewise becomes optically negative by nitration and complete acetylation (triacetyl cellulose), i.e., by the esterification of the polar OH-groups. It may therefore reasonably be presumed that it is the acetyl side chains of the chitin which cause the negative birefringence. It is nevertheless a curious fact that for chitin only one acetyl group per glucose residue is required for this, whereas three are necessary in cellulose; presumably, therefore, the amino group of the glucosamine also tends to produce negative birefringence.

The negative intrinsic birefringence of chitin does not hamper the approach to the micellar texture of chitinous composite bodies by polarizing optics if the imbibition agents used are liquids whose refractive index is below 1.48, i.e., water or glycerol. It should, however, be realized that the determination of the micellar orientation does not then take place on the basis of the positive intrinsic anisotropy of the submicroscopic frame of the membrane, as in cellulose, but rests on the positive rodlet birefringence of the chitin skeleton.

With chitin as the last, we now turn aside from the group of frame substances in the carbohydrate class and turn to that important category which, chemically, shows greater affinity to protoplasm, viz., the frame proteins.

d. *Silk (Silk Fibroin)*

Microscopic and submicroscopic structure of silk. A cross-section of the cocoon thread of the silk-moth (*Bombyx mori*) reveals two halves in mirror symmetry, which owe their existence to the paired silk-glands. These produce two discrete fibroin threads of sectoral cross-section which are covered with a layer of sericin (Fig. 141). The regular structure as seen in Fig. 141a is apparently disturbed where the threads cross in the cocoon, which would go to show that the thread is still plastic when it leaves the silkgland. A finer structure is revealed both by the sericin layer and by the fibroin threads (OHARA, 1933a). On the outside is a very weakly birefringent, almost amorphous membrane of sericin, under which comes a strongly birefringent layer of fibroid texture. The sericin layer is separated from the fibroin threads by an isotropic lamella. It is here that the sericin becomes detached from the fibroin when the silk is degummed in a bath of boiling water. Two degummed silk threads are then formed from every cocoon filament or raw silk thread. Thus the difference between raw silk and ordinary degummed silk is that the former is still surrounded by the sericin cortex, though admittedly this has as a rule suffered considerable mechanical damage.

Fig. 141. Fine-structure of silk. *a*) Microscopic cross-section through the cocoon filament (after OHARA 1933a); *b*) submicroscopic structure of the fibroin thread. 1 Skin and 2 cortex (fibroid texture with tangential scattering) of the sericin layer. 3 Skin, 4 cortex (fibroid texture with radial scattering) and 5 central zone (fibrous texture) of the fibroin filament.

The fibroin filaments, which are now to form our main topic, have, according to OHARA, three zones which are optically distinguishable, i.e., a central zone of fibrous texture, a cortical layer around this of fibroid texture and, finally, at the outside a skin layer. This is only slightly anisotropic, yet its texture is apparently slightly

fibroid. It is interesting to note that here the scattering — i.e., deviation of the optically positive submicroscopic fibroin rodlets — is not tangential, as in the sericin layer or in cellulose fibres, but radial (see Fig. 141b). It seems that in the process of degumming the character of the scattering in the coating layer changes from radial to tangential, for, after the hot water treatment, the large axis of the index ellipse lies tangentially. The scattering of the fibroid texture of the cortical layer is likewise

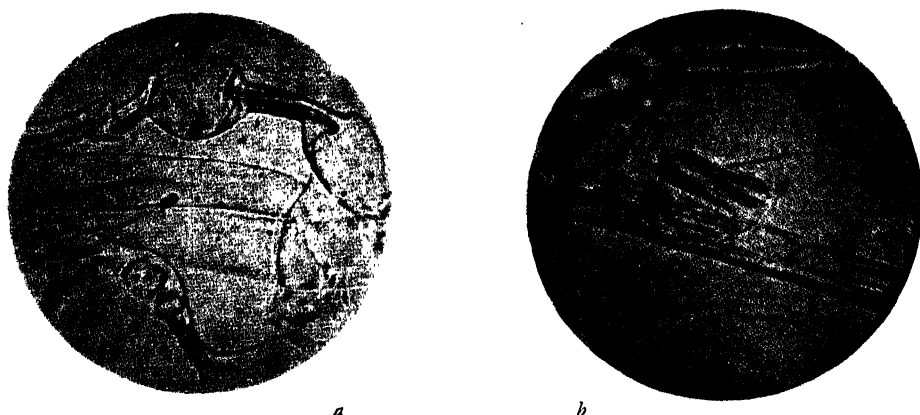



Fig. 142. Fine-structure of silk (after OHARA 1933b). *a*) Beading with $Ca(NO_3)_2$ solution; *b*) fibrillar formation with hypobromite.

radial. Thus in a cross-section through the cocoon threads, sericin and fibroin are easily distinguishable by their different optical behaviour in a polarizing microscope in which a selenite test plate has been inserted, in that the sericin wrapping produces a negative, and the fibroin cortex a positive spherite cross (cf. Fig. 68). In the cross-section the central zone appears to be isotropic.

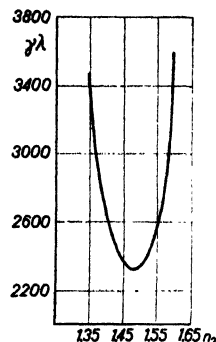
The zoning of the fibroin filament is brought out clearly by dichroitic gold and silver staining. In conformity with its fibrous texture, the central zone exhibits pronounced dichroism; in the cortical zone, on the other hand, with its far inferior orientation, the coloration is not dichroitic, and in the coating layer there is none at all. According to OHARA (1933a), this is how the fibroin filament laminates as a result of coagulation: The coating layer is the first to coagulate on leaving the silk-gland, before there is any opportunity for a submicroscopic structure to be formed in some order. A little later, the cortical layer, the fibroin thread molecules of which are already to some extent orientated, coagulates. Finally, the fibroin mass in the central zone remains plastic for a longer period and the chain molecules of the silk fibroin are all able to orientate with parallel axes before they combine to form a micellar frame. As rayon filaments often display a similar structure (PRESTON, 1933), OHARA's hypothesis is attractive, but it should be pointed out that, contrary to natural silk, the cortical part of viscose is submicroscopically better orientated than the central part of the filament ("skin effect" according to PRESTON, 1933). In rayon, the stretching process brings about the orientation of the peripheral region, that process orientating the already coagulated skin part, whereas the thread molecules of the as yet uncongealed mass at the core of the filament are not effectively held by the orientating forces, owing to their mobility. Hence the optical conditions prevailing in natural silk which conflict with this interpretation must be explained in some

other way. As I see it, only the central portion of a liquid that issues from a capillary flows at maximum velocity, this velocity decreasing parabolically towards the capillary wall, there becoming nil (FREY-WYSSLING, 1932). On this hypothesis, the thread molecules of the silk fibroin in the middle of the filament are orientated in parallel, whereas in the cortical region they are orientated obliquely to the filament axis owing to the velocity gradient, that is to say radially, as has indeed actually observed. This would explain the unusual radial scattering in the cortex of the fibroin filament.

Since, like vegetable bast fibres, the silk fibroin filaments possess a central portion of fibrous texture and a skin with pronounced micellar scattering, their swelling and hydrolysis phenomena are similar to the cellulosic walls of fibre cells. Thus OHARA (1933b) finds a beaded appearance in silk similar to that found in cellulose fibres, when the central portion, expanding powerfully, are pressed through weakened spots of the skin layer (Fig. 142). Furthermore, the central zone can be split up by bromine lye (hypobromite) into fibrillae, which then disintegrate into short bundles of fibrillae, as in cellulose fibres. These facts are important, in that they imply that, contrary to LÜDTKE's statement (1936) respecting cellulose fibres, beading and hydrolytic disintegration perpendicularly to the fibre axis do not depend upon any membrans across the fibre, for there can be no question of the formation of any such hypothetical segmentation during the nascence of the silk thread.

Fig. 143. Rodlet birefringence curve of the fibroin thread (after OHARA 1933a). Ordinate: retardation $\gamma\lambda$ in Å. Abscissa: refractive index n_2 of the imbibition fluid.

As with other fibrous structures, the intermicellar system of silk must be considered as composed of submicroscopic capillaries, all connected by longitudinal cleavage. In tussah silk (HÖHNEL, 1887, p. 145) these voids are, under certain circumstances, sometimes big enough to be microscopically visible as small air ducts. It is probable that, as in cellulose, there is amorphous fibroin besides the crystalline micellar strands, subdividing the intermicellar system.

The value of the optical anisotropy of silk fibroin is also strongly reminiscent of cellulose. By BECKE's method, OHARA (1933a) finds n = 1.584 for the highest refractive index in sodium light in the fibre direction and, perpendicular to it, n = 1.529, from which the high birefringence Δ n = 0.055 results. HEGETSCHWEILER (1947) reports as Δ n = 0.0506. If imbibed with liquids of increasing refractive power, the fibroin threads show distinct signs of rodlet birefringence. OHARA (1933a) describes an elegant rodlet birefringence curve possessing for yellow mercury light (λ = 576 mμ) a minimum at n_D = 1.472 with retardation $\gamma\lambda$ of + 237.5 mμ (Fig. 143). It follows from this that the submicroscopic silk fibroin rodlets have a positive intrinsic birefringence. The fibroin filaments examined by OHARA are approximately 5 μ thick; and, computed from the retardation given, the intrinsic birefringence comes to Δ n = $\gamma\lambda/d$ = 237.5 : 5000 = 0.048. As this figure is 13 % lower than the birefringence obtained by direct measurement, i.e., $n\gamma - n\alpha$ = 0.055, the inference is that about one-tenth of the thickness of the thread is occupied by isotropic not crystallized fibroin. As, however, the minimum of the curve, which indicates the refractive power of the object, is at 1.472, whereas both $n\alpha$ = 1.529 and $n\gamma$ = 1.584 as measured were substantially higher, there is here an incongruity which must be cleared up before any quantitative conclusions are drawn. Probably, as with chitin

(see page 194) and cellulose (FREY–WYSSLING, 1936b), the refractive power of the micellar system in silk fibroin is changed during imbibition by adsorptive processes.

Molecular structure of silk fibroin. It is to be presumed from the many points of similarity between silk fibroin and cellulose that the microstructure of these two fibrous materials is based on the same principle. In fact, fibroin, like cellulose, consists of primary valence chains (MEYER and MARK, 1928) of unknown length, which combine to form ordered lattice regions of extreme rodlet shape and of optically positive character. This provides one of the most striking examples of how a common morphological fundamental principle in the submicroscopical region produces configurations possessing similar microscopic and macroscopic properties in substances even of entirely different chemical composition.

Fig. 144. Molecular structure of silk fibroin.

For, chemically, the fibroin thread molecules, as polypeptidal protein chains, are not in the least akin to cellulose. They are composed to the extent of roughly 60 % of glycine and alanine, furthermore containing tyrosine and other α-amino acids. In profile these chains present the familiar picture of the zig-zag line with consecutive CO, NH and CH groups (Fig. 144a). ASTBURY (1933a) calls the distance between neighbouring chains "backbone spacing" and it measures 4.5 Å. It may therefore be said that the spatial depth which a chain requires in the plane of the zig-zag line, or the "backbone thickness", amounts to 4.5 Å. The side chains of the CH groups are not seen in the profile view of Fig. 144a, as they stand off, like ribs, perpendicular to the backbone plane, suggestive of a vertebrate skeleton. The thread molecule has, therefore, to be seen from the front to get the side chains in their proper place (Fig. 144b). Thus the kinked chain presents itself as a straight line with perspectively shortened valence bonds, while the side chains lie in the plane of the drawing. The glycine residue can scarcely be said to have a side chain, as in this case it is represented merely by the insignificant H atom; but with the alanine residue it consists of a methyl group and with the tyrosine residue of a parahydroxyphenyl ring as well. The side chains are not fitted in pairs like actual ribs but point alternately to left and right on consecutive CH groups, with the result that every two neighbouring amino-acid residues together form a morphological unit, which in the X-ray diagram becomes the fibre period. In silk fibroin it amounts to 6.95 Å. From this it may be concluded that the length of each backbone segment, or in other words the extension of each individual amino acid member, is 3.5 Å. This length is quite irrespective of the nature of the amino acids in the primary valence chain. Thus all the entirely different components, glycine, alanine and tyrosine, represent, as members of the chain, sections of the thread molecule of exactly the same length (ASTBURY, 1933b). They can therefore be interchanged without thereby causing any alteration in the fibre period or the backbone thickness.

The elongation of the side chains is quite a different matter, for this, as may be seen, is very unequal. In order to preserve rigid regularity in this respect as well,

Meyer and Mark (1930) assumed that only glycine and alanine residues interchanged in crystallized silk fibroin, while the other amino acids produced by hydrolysis are interposed amorphously between them as intermicellar substance. It does not seem likely that this view can be maintained, for up to the present it has not been possible to define an undoubted elementary cell of the crystal lattice as in cellulose (Kratky and Kuriyama, 1931; Sakurada and Hutius, 1933; Brill, 1943). The reason may be a certain irregularity caused by the side chains. Kratky and Sekora (1944) treated silk fibroin with iodine and found by X-ray analysis a new period of 70 Å parallel to the fibre axis. As it is likely that the iodine is introduced into the tyrosine residue, every 20th amino acid of the polypeptidic chain should be tyrosine. By chemical analysis Bergmann and Niemann (1938) find that out of 16 amino acid residues one is tyrosine. It is therefore probable that tyrosine belongs to the crystallizing polypeptide chains. The primary valence chains are held together by hydrogen bonds (see p. 99) to form a chain lattice (Brill, 1941).

Whereas the similarities between the physical properties of silk and cellulose fibres (tensile strength, swelling, optics, etc.) derive from their common molecular chain morphology, their distinguishing characteristics reside primarily in their chemical properties. Thus the —CO—NH-bonds of fibroin hydrolyse far more readily than do the glucosidic bonds >CH—O—CH< of cellulose, for which reason silk is exceedingly sensitive to a great variety of ostensibly mild treatments (e.g. boiling, contact with salts). Silk is not neutral, like cellulose, in the presence of dyestuffs, but behaves like a weak acid, so that basic dyes (such as methyl green) are easily absorbed. This might be due to, say, the tyrosine side chains, as hydroxyphenyl groups behave as though slightly acid. Nevertheless, after boiling for two hours, at 100°C (Ohara, 1933a), the reaction of fibroin changes to the basic, probably through hydrolysis of acid amide bonds, and the silk so treated can then be dyed by acid dyestuffs, such as acid fuchsine.

e. *Horny Substances (Keratin)*

Microscopic structure and optics of hair. The great technical importance and the remarkable elastic behaviour of wool and other hairs were the incentive prompting research respecting keratin.

Microscopically, the hairs consist of three layers, viz., a very thin, scaled and unpigmented upper pellicle (epidermicula), a thick, fibrous cortical layer containing the pigment and a parenchymatous pith. Sometimes there is no pith, as in Merino wool. The surface skin, covering the cortex with scales ringshaped or like roofing tiles, may likewise disappear owing to mechanical chafing, and yet the elastic and optical properties of the hair will not radically change. Their source is, therefore, the keratin fibre cells of the cortex, which consist of numerous tonofibrillae orientated in parallel. In the electron microscope the fibrillae can be seen to unravel into still finer subfibrillae (Reumuth, 1942). The length of keratin fibres varies between 50 μ and a few millimetres, in sheep being about 80 μ (Höhnel, 1887). The fibres are as a rule flattened, so that the radial diameter is the narrower. Although a hair may under certain circumstances appear optically to be entirely homogeneous, cytologically it is not comparable to a single bast fibre, but to multicellular strands of bast fibres consisting of relatively short fibre cells, as they occur in Monocotyledones (sisal, Manila hemp, etc.).

Unlike cellulose fibres, horn fibres are extremely elastic. In cold water a hair

can be stretched reversibly 50 to 70 %, whereas bast fibres of good fibrous structure break upon being elongated to only a few units per cent. The elastic elongation of the hairs is especially impressive under the polarizing microscope (POCHETTINO, 1913). Although the cross-sectional area of the hair decreases owing to the elongation, the retardation of light increases considerably, which is apparent by the sharp rise in interference colours. It is a fascinating spectacle to watch the polarizing colour of

Fig. 145. *a)* β-keratin after ASTBURY (1935*c*). *b)* Folding of the polypeptide chain after ASTBURY & BELL (1941); R side chains.

weakly pigmented (fair) hair reversibly waxing and waning in intensity as the hair is stretched and released. The conspicuous change in optical anisotropy is to be explained by birefringence of stress. Whereas photo-elastic effects of the kind, however, are usually brought about by slight changes of distance in the crystal lattice which are not detectable by X-ray (WIENER, 1926b), the molecular frame of keratin is completely re-formed during the latter's elongation.

In curly wool the stretched outer side takes basic dyes (Janus green, neutral violet, pyronine) more easily, and has a lower refractive index in a radial direction, than the inner side of the curl (OHARA, 1938, 1939).

Molecular structure of keratin. ASTBURY (1933c) has demonstrated that elongated hairs produce quite a different X-ray diagram from that of unextended hairs. The difference is especially evident when the elongation takes place in a vapour-saturated chamber at 100° C, when about 100 % elongation can be attained. The X-ray picture shows the distance between the members of the chain to be 3.38 Å. This tallies well with the chain period of silk fibroin, viz. 3.5 Å and it may therefore be assumed that elongated primary valence chains of polypeptide thread molecules are also present in stretched wool. As the fibre period in unstretched wool is 5.06 Å, there must be some other modification of keratin, which ASTBURY designates as α-keratin, distinguishing the modification like silk fibroin of elongated wool as β-keratin. By folding the polypeptide chain diagram, he also succeeded in deriving α-keratin from β-keratin, reasoning that by the mutual attraction of two NH and CO groups sepa-

rated by five valence bonds, pseudo-diketopiperazine rings are liable to be formed. Taking into account the rules of distance, the fibre period of α-keratin for a chain thus folded comes to 5.06 Å. As the diagram shows (see Fig. 145a), the chain length is doubled at full stretch (100 % elongation).

The ingenious theory of folding to form piperazine rings is confronted with steric difficulties; for the side chains R, which point in the same direction, come so close together that they hinder each other spatially. ASTBURY and BELL (1941) have therefore drawn up a new folding diagram for the β-α transformation, which satisfies the following conditions:

1. The α-form must be about half as long as the β-form. 2. The density must remain practically constant. 3. The folds must repeat at a distance of about 5.1 Å. 4. The side chains must stand out alternately on one side and the other of the plane of the fold. 5. The folds must be nowhere so sharp as to have insufficient room for the side chains.

This diagram is reproduced in Fig. 145b. Side chains pointing upwards are marked R enclosed in a full-line circle and those pointing downwards by R within a dotted circle. The side chains standing on the same side form groups of three, which in the diagram appear as the angles of the triangles indicated.

The R side chains are particularly important. If hairs elongated in vapour of 100° C are dried in the stretched state, the elongation loses its reversibility and is retained. The side chains of neighbouring polypeptide chains enter into spatial relationship and connect the primary valence chains to a kind of grate (Fig. 146). The distance between the bars of the grate is 9.8 Å; hence the side chains, which at intervals of 3.38 Å stand off more or less perpendicularly from the primary chains to the right and left, should have half that length. The thickness of the grate corresponds to the backbone thickness of the stretched, zig-zag polypeptide chains and is therefore 4.5 Å.

Glutamic acid, arginine and cystine are among the most important products of the hydrolysis of wool (see Fig. 89). Assuming amidic linking between glutamic acid and arginine, there will be a kind of rung linking two primary valence chains, as represented in Fig. 146b. Retaining the tetrahedral angle, this side connection would be about 12.5 Å long. It is, therefore, of the order of magnitude of the length found by X-ray measurement, viz., 9.8 Å, for it is quite conceivable that the chains may somehow be shortened.

Cystine is the most interesting of the three. This contains two amino acid residues united by a sulphur bridge. It is assumed (ASTBURY, 1933c; MARK and PHILIPP, 1937) that such sulphur bonds primarily hold the polypeptide chains together in keratin, for sulphur plays a similar part in vulcanized rubber. It connects the free polyprene chains of the raw rubber laterally, in this way producing a molecular frame, and thus enhances the elastic properties of the raw rubber, while its plasticity deteriorates. If too many sulphur bridges are introduced, however, the material will lose its elasticity, being "vulcanized to death", and hard rubber or ebonite results. Now there is some analogy between raw rubber and vulcanized rubber, on the one hand, and muscular myosin free from sulphur (page 206) and sulphurous keratin, on the other. By way of comparison, therefore, the tonofibrillae have been termed "vulcanized" muscle fibres, which would explain the loss of contractility and their great strength.

Despite illuminating comparisons such as these, which are very helpful to a

qualitative interpretation of many interesting properties, there remain many serious quantitative obstacles to a complete comprehension of the submicroscopic structure of keratin. Above all, the length of the cystine molecule does not agree with the X-ray evidence as to the length of the keratin side chains. As is apparent from Fig. 146b, the sulphur bridge is by no means long enough to span the distance of 9.8 Å from primary chain to primary chain. Hence the molecular frame cannot be as simple

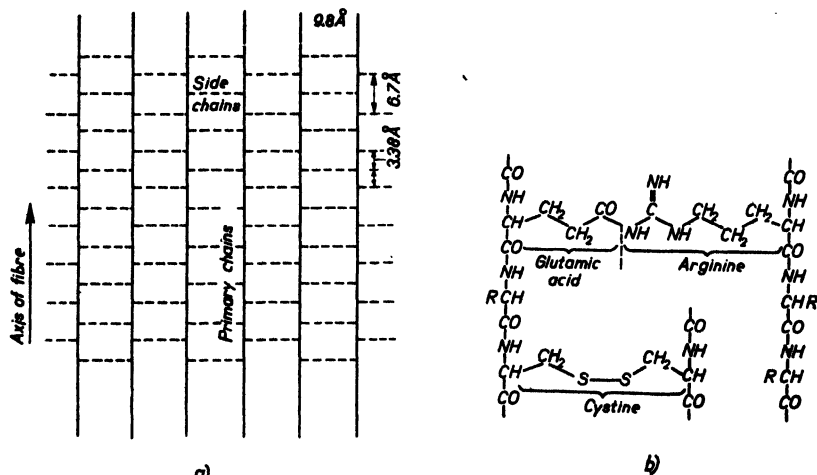



Fig. 146. *a*) Keratin frame as lattice grid; *b*) side chains of keratin.

and orderly as it is represented in Fig. 146a; possibly, therefore, some other amino acid besides cystine — say glutamic acid — assists in bridging this great distance.

There is, however, another reason why the molecular frame is unlikely to be of simple structure. ASTBURY (1933c) advances plausible arguments to show that, in the re-transformation of the β-keratin of elongated hairs into the folded α-keratin, side bridges must be broken off. This, with a planar molecular frame, would be avoidable only if all the parallel zig-zag polypeptide chains could be folded simultaneously perpendicular to the projection plane of Fig. 146a without involving the detachment of the punctuated side bonds. If, however, the primary valence bars are mutually linked in various directions, the individual polypeptide chains can no longer be folded without breaking up the side-chain bonds.

It is very significant that ordinary water is capable of disrupting the bonds in question in the case of β-keratin; for a hair elongated to double its length and then dried has only to be placed in water to regain its reversible elasticity. This means that drying brings about only temporary, and not permanent, set. Nevertheless, if a hair elongated 100 per cent, is left for half an hour in a vapour bath, it loses the capacity to contract again to its original length, being now permanently set and retaining this imposed length even when wetted in what is known as "permanent set". This fact is put to a banal utilitarian use in the hairdressing profession, for it is only when the hairdresser succeeds in imparting permanent set to the β-keratin produced at the curved places in the hair that he can claim to have provided a „permanent wave". The permanent setting of the β-keratin is said to be achieved by the prolonged action of the vapour, whereby so many strong bridges are laid between the keratin chains that hot water is subsequently unable to disrupt them.

ELÖD, NOWOTNY, and ZAHN (1940) oppose ASTBURY's theory that keratin contains grate frames connected by sulphur bridges in the side chains. Treatment of the wool with metallic mercury will convert 50 per cent of the keratin sulphur to HgS. Removing half of the –S–S–bridges should weaken the molecular frame, involving modification of the properties of the wool. This, however, is not the case and these investigators therefore assume that it is not the side chains which build up the frame, but as in silk fibroin, hydrogen bonds (see Fig. 96) between the primary chains in the backbone planes (NOWOTNY and ZAHN, 1942). The side chains, they say, stand perpendicular to the planes of the frame and it is therefore of no consequence if they differ in length. It is assumed that the gratings form a laminar structure parallel to the surface of the hair or nail.

If a hair which has been elongated to 100 % and temporarily set is placed, free, in a vapour bath for a short time, it will contract, not only to its original length, but considerably further; obviously, supercontraction takes place. This fact implies that the polypeptide chains in the α-keratin are not entirely free and independent of each other; rather, it would seem that they too are mutually stabilized by certain bridges. Apparently, however, the treatment mentioned breaks up this linkage and enables the polypeptide chains to fold far more than before. Restrictive lateral bridges of this kind are also made responsible for the fact that a hair is only 50–70 % extensible in cold water; they slacken in hot water and the polypeptide chains can therefore be fully stretched (about 100 %).

The fact, then, with keratin is that if it is exposed for a short time to the action of hot water or vapour, connecting bridges between protein chains are broken down. Since dilute caustic soda solution similarly loosens the set chains, this might be a case of hydrolytic decomposition of acid amide bridges. Yet the selfsame treatment, if more prolonged, will facilitate the formation of new, stronger bonds. In view of the theory regarding the structure of cytoplasm developed in this book, this behaviour appears to me to be very significant, as it shows how readily the frame of proteins can be destroyed and built up again. Seeing that hot water suffices to initiate this process in keratin, it is not difficult to imagine how, in the far more labile cytoplasm, the protein thread molecules are constantly forming new combinations and side bonds, while others are continually being broken down, so that a quite definite molecular frame is always in existence, despite the apparent liquid state of the material.

Fine-structure of finger nails. Finger nails are built up of submicroscopic fibrillae. X-ray analysis shows that the keratin fibrillae run, not parallel, but perpendicular to the longitudinal axis of the nail (DERKSEN, HERINGA, and WEIDINGER, 1937). As in the elongation growth of the plant cell wall, therefore, the micellar texture is orientated perpendicular to the direction of growth. The alignment of the micellar strands, therefore, is not a passive process due to the forces of growth pushing the nail forward; there are special formative forces at work, building up submicroscopic textures with due regard to their future functions.

Feather keratin. Not all the horny substances are naturally in the state of α-keratin. Instead of the fibre period for mammalian hair, viz., 5.06 Å in the direction of the primary chain, that of quills in the unextended state is 3.1 Å (ASTBURY and MARWICK, 1932). By elongation it can be increased continuously and reversibly to 3.3 Å but, if subjected to further elongation, the quill breaks. From this fact it may be concluded that the polypeptide chains in quill keratin are stretched approximately in the same way as in elongated hairs or in silk fibroin. The fact that the length of the members

of the primary chains is neither 3.38 Å nor 3.5 Å is said to be due to slight corrugation (so-called "primary folding") of the polypeptide chains in the feather keratin, owing to a certain interaction of the side chains. This slight primary folding is also supposed to be responsible for the shortness, as compared with silk fibroin, of the amino acid residues of β-keratin. The far sharper kinks in the α-keratin chains are distinguished from this slight corrugation as "secondary folding". Thus the scleroprotein of quills is a modification of keratin in which there is no secondary folding. The keratin primary valence chains are therefore used by the animal body for the building of the horny tissues, either heavily folded, or in an approximately stretched state.

f. *Sinews (Collagen)*

Sinews and demineralized bones consist of the gelatinous protein collagen. Glue and gelatine are relatively little changed decomposition products of this insoluble frame substance which have become soluble in hot water owing to slight hydrolytic degradation. Sinews and elongated gelatine produce the same X-ray pattern (GERNGROSS and KATZ, 1926). It shows 8.4 Å as the fibre period which, divided among three amino acid residues, shows the length of the members of the primary chain to be 2.8 Å. Moreover there are two interferences on the equator of the diagram, which correspond to 4.65 Å (backbone thickness of the primary chain) and 10.0 Å (length of the side chain). The resemblance to the conditions on β-keratin is striking; only as compared with the amino acid residues of silk fibroin and of keratin, the primary chain period of 2.8 Å would appear to be rather short. This may be due to the presence in the collagen of other amino acids (KÜNTZEL, 1941), viz., about 34 % of proline and oxyproline in addition to 25 % of glycine. The many five-membered rings cannot, of course, all act as chain end groups (see Fig. 89); they must surely be built into the primary chains (Fig. 147), causing considerable primary folding (ASTBURY, 1940). Furthermore, collagen, like the other frame proteins, appears to be built up according to the diagram of polypeptide chains of indeterminate length.

Fig. 147. Diagram of a gelatine chain.

Tautomeric rearrangements would also help to explain the shortening of the members, for if within the stretched chain the hydrogen of every second NH group were to be transferred to the neighbouring CO group, double bonds –N=C(OH)– would be formed which entail the stereoisomeric possibilities of the cis and trans configuration. Now if the bed-form cis position is assumed, the members of the chain are shortened to the experimentally ascertained period, 2.86 Å (cf. HALLE, 1937; CHAMPETIER and FAURÉ-FREMIET, 1938).

There is a similar small fibre period of 2.9 Å in elastoidin (CHAMPETIER and FAURÉ-FREMIET, 1937), which is the frame substance of the fin rays of *Selachii* (FAURÉ-FREMIET, 1936). Its optical, thermal and swelling properties are exactly comparable to those of collagen, from which elastoidin is

distinguished by slight chemical differences in resistivity to trypsin and by sulphur content.

Optically, sinews and gelatine filaments are positively uniaxial as referred to the fibre direction, i.e., the same as silk and hairs. Rodlet birefringence is also distinctly apparent if the sinews are tanned before imbibition (KÜNTZEL, 1929); the latter are also very liable to swell in the presence of most imbibition liquids, or to shrink (e.g., with xylene). Collagen behaves very peculiarly in tanning; for whereas the optical character of the sinews is retained with mineral tanning materials (chromic salts) and formol, it is reversed and becomes negative with pyrogallic tanning agents (tannin, sumach) and other phenols (trinitrophenol) and aldehydes (eugenol, cinnamic aldehyde). JAEGER (1944) finds that human sinews fixed in formol likewise possess negative intrinsic birefringence. SCHMIDT (1934a) imagines that the optical negative reaction is brought about by orientated adsorption, as the non-tanning univalent phenols and aldehydes may be washed out again, whereupon the normal optically positive reaction returns. Personally, I am inclined to believe that it is rather a matter of chemical changes in the side chains. Tanning depends upon the permanent connecting of one polypeptide chain molecule to another by strong side-group linkages. Moreover, the pyrogallic tanning agents must thereby change the polarity of the side groups in a manner similar to what takes place in the nitration or acetylation of cellulose. In view of the lability of many side chain reactions of the polypeptide chains, it is not surprising that washing out of the non-tanning phenols should easily upset the chemical changes brought about by trinitrophenol, eugenol, and so on.

Rodlet birefringence and X-ray analysis provide evidence for the submicroscopic fibrous structure of sinews. Nowadays fibrillated sinews can be examined in the electron microscope. The smallest microfibrillae into which they can be split up reveal a peculiar segmentation (WOLPERS, 1944, see Fig. 151a) ,which will be discussed when treating the striped muscle fibre (p. 210).

It is not only the strange optical behaviour of sinews which has for long attracted attention (v. EBNER, 1894), but also their remarkable swelling power. In water they swell by 50 % in thickness, which, as X-ray evidence shows, involves expansion up to 35 % of the crystal lattice (KÜNTZEL and PRAKKE, 1933), while the fibre period remains unchanged. Hence the swelling is not intermicellar as in cellulose, but intramicellar, inasmuch as the individual primary valence chains are pushed apart. This explains why the swelling of sinews may assume unprecedented dimensions. In dilute acids and alkalies, which obviously completely hydrolyse the side chain bonds, they are liable to swell 550 % in thickness, though admittedly they at the same time shorten by 30 %. Despite this shortening, the increase in volume due to the infiltration of fluid may amount to as much as 4500 % (KÜNTZEL and PRAKKE, 1933).

Reduction in length becomes more strikking when the sinews are placed in hot water (60 to 70° C). Suddenly, while thickening, they contract, at which moment birefringence and X-ray diagram vanish. This unusual reduction in length imparts rubber-like elasticity to the sinew. After careful elongation the X-ray diagram reappears and continued stretching will finally restore and establish the inelastic collagen fibre. All this resembles the behaviour of rubber which, unstretched, produces no X-ray diagram, but does show up interferences after it has been considerably stretched. MEYER and MARK (1930) point out another interesting property common to both materials. If the contracted sinews or unstretched rubber be frozen in liquid air and

the objects be then smashed, they crumble to a friable mass, like sand; whereas under similar treatment native sinews or elongated rubber will split up into a fibrillar mass. From this it may be inferred that the polypeptide chains of the collagen fibres contract, as in β-keratin, and fold up. But whilst folding in the $\beta \rightarrow \alpha$-keratin conversion remains within precise limits, with collagen it is erratic and so violent that the protein chains shrivel up completely. Evidently, therefore, the impulse of the polypeptide chains to shorten in the free state is very widespread and, if means are found to make this process reversible and to regulate it, a model will be provided for the contractile muscle fibres.

g. *Muscle Fibres (Myosin)*

Although muscle fibres do not belong to the category of frame substances, we shall nevertheless discuss them here because, as SCHMIDT (1937a) demonstrates, there is no decided contradistinction between the "inanimate" metaplasmatic formations (such as tonofibrillae) and mesoplasmatic cell constituents (like myofibrillae) which, in spite of being irreversible differentiations of cytoplasm, exhibit their own specific active metabolism and transformation of energy. Preeminently, submicroscopic morphology supplies evidence whereby it can be shown that only very slight structural differences exist between the various fibrous protein systems in the molecular world which are responsible for the manifestation of intense, moderate or no viability in these plasmic derivatives. There are no well-defined limits and it is difficult to say at what degree of mobility, a derivative of the protoplasm may be said to be invested with Life.

Optics of striped muscle fibres. The safest way to assess the microscopic structure of the highly differentiated striated musculature is between crossed nicols (VLÉS, 1911; v. MURALT, 1933; SCHMIDT, 1937a). This circumvents many sources of error, such as the complicated diffraction phenomena of striated systems (PFEIFFER, 1942b; LANGELAAN, 1946), or the changes in structure so greatly, though wrongly, feared in the fixation of the tissue. The optical polarization pattern of living muscle fibres upon which the following description is based anyhow agrees largely with the dyeing of well fixed muscles.

The muscle fibre, 10 to 100 μ in width, which is enclosed in a thin skin, the sarcolemma, disintegrates into optically resolvable fibrillae about 1 μ thick and at roughly 0.5 μ distance from each other. The visible fibrillae consist of bundles of parallel submicroscopic elementary fibrillae, which HÜRTHLE (1931) assumes to be from 50–100 Å thick (Fig. 149). The sarcoplasm, which surrounds the fibrillae on all sides, lies in between the myofibrillae. Essentially it should consist of muscle albumin, or myogen, while the fibrillae are identical with, in the main, the muscle globulin, or myosin. The sarcoplasm is always isotropic, but myofibrillae transmit light in the polarizing microscope and exhibit the familiar segmentation in bright, so-called Q sections and dark, very weakly birefringent I sections. There are accumulations of nucleic acids of the adenyl nucleotid type in the semi-isotropic I sections (CASPERSSON and THORELL, 1941). The remarkable part of this structure is that all the fibrillae of a muscle fibre, though mutually independent, have their luminous and dark segments at exactly the same level, with the result that the entire fibre is evenly segmented or striated.

The coincidence of the strongly and weakly birefringent sections is due to the division of the individual fibrillae after the segmentation of the original mother

fibrillae has taken place. Despite the conspicuous optical differentiation, the fibrillae are not transversally subdivided, but run in uninterrupted succession through the entire length of the fibre. Their cleavability, which betrays no mechanical inhomogeneity at the boundaries of the segment, is an argument in favour of their uniformity. Further, very young fibrillae are uniformly birefringent (later the striation appears gradually differentiated from the middle towards the extremities), or the cross striation may disappear in explanted skeleton muscle cells through dedifferentiation (SCHMIDT, 1937a, pp. 215, 223).

Whereas the fibrillae may thus be taken to be continuous, the sarcoplasm appears to be subdivided by transversal partitions; for, in the centre of the dark section I there is always a narrow band, easily identified by staining, which shines brightly between crossed nicols and which is denoted as the Z stripe (Fig. 148). It is conceived to be a cross membrane, intergrown with the sarcolemma, the myofibrillae thrusting through it without hindrance. When the muscles contract, these places thicken only a little, so that the sarcolemma festoons, as it were, the tightened muscle fibres.

The contraction is not limited to the strongly birefringent Q section; the I sections also shorten, though to less extent. The retardation in the fibres decreases considerably during contraction, notwithstanding of the appreciable increase in thickness; the optical term for this is negative fluctuation. The fact established by v. MURALT (1932) that negative fluctuation also occurs with isometric contraction — i.e., when, during excitation, the muscle is forcibly held to its original length — is of the utmost importance.

Submicroscopic structure of myofibrillae. Besides intrinsic birefringence, which is manifested as birefringence of flow in myosin solution (v. MURALT and EDSALL, 1930), the myofibrillae exhibit distinct rodlet birefringence (STÜBEL, 1923). It follows from this that the fibrillae are not of uniform structure, but are of the class of rodlet composite bodies. BOEHM and WEBER (1932) produced composite bodies of the kind artificially by injecting myosin solutions into water. The resulting filaments displayed the same optical properties, both qualitatively and quantitatively, as the Q sections of the myofibrillae (WEBER, 1934). It is very surprising to find how well the measured birefringence agrees with that calculated from WIENER's formula (see p. 59). for the assumptions of WIENER's composite bodies are hardly applicable to hydrophilic micellar systems. Before all, the theory requires that there should be a well-defined phase boundary between the rods and the imbibition liquid, which there cannot be with a swellable protein which adds on water molecules to its macromolecular chains. There is another assumption which is more to the point in this case, it being that the submicroscopic rodlets have practically unlimited length. WEBER, it is true, assumes a particle length of only 500 Å and WORSCHITZ (1935) has X-ray evidence for lengths up to 2050 Å, but no reliance can be placed on X-ray determinations of particle length with dimensions beyond 500 Å (see FREY-WYSSLING 1937a, page 376). It may therefore be assumed with equal justice that the optically identified rodlets are bundles of primary valence chains which run parallel through the myofibrillae to unknown distances.

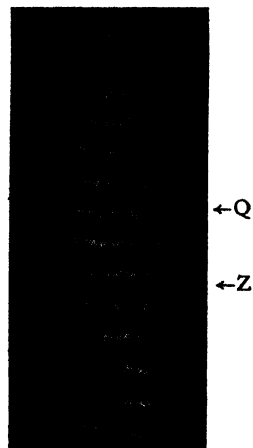



Fig. 148.
Laterally striated muscle fibres between crossed nicols (after SCHMIDT 1937*a*). Wide Q sections and narrow, weakly luminous Z stripes.

Following HÜRTHLE (1931), one might describe these bundles as elementary fibrillae, but they need not necessarily be independent; on the contrary, the possibility exists that individual primary valence chains of a bundle may pass over to neighbouring elementary fibrillae. By WORSCHITZ' photometer evaluation the bundles should have an average thickness of approximately 75 Å. V. ARDENNE and WEBER (1941) find with myosin precipitations in the electron microscope very fine filaments of 50–100 Å diameter.

Fig. 149. Submicroscopic structure of cross striated myofibrillae. E = elementary fibrillae of primary valence chains; S = sarcoplasm; Q = anisotropic, I isotropic section; Z = anisotropic cross stripe. Scale: Length 100 times smaller than width.

X-ray analysis gives us some information on the inner structure of elementary fibrillae. Myosin filaments produce the same X-ray diagram as relaxed muscles (BOEHM and WEBER, 1932) which proves the identity between the fibrillar substance and myosin. Model experiments can therefore be carried out with myosin films and it is in this way that ASTBURY and DICKINSON (1935a) found that the X-ray picture of muscle protein is exactly similar to that of keratin. The $\alpha \rightleftharpoons \beta$-keratin conversion can be attained by elongation, but in the relaxed muscle it is not the stretched β-form, but the folded α condition. True, the modification of myosin to the β-form can also be forced upon the muscle by artificial extension (ASTBURY and DICKINSON, 1935b), but the α-form always occurs in the natural state. Hence it must be assumed that the polypeptide thread molecules in the relaxed muscle run, as in unstretched hairs, in folded chains parallel to the fibre through the fibrillae. Optically, uniformly birefringent myofibrillae should appear from this in their entire length, as we know them in the smooth muscle fibres. This is where the X-ray method is at a distinct disadvantage compared to polarization optics, for it fails to distinguish the better orientated Q sections of striped muscles from the almost isotropic I bands. To understand the zoning it is necessary to suppose that the continuous primary valence chains in the scarcely birefringent I sections forfeit their fibrous structure and that the optical anisotropy is replaced by statistical isotropy, e.g., by a zig-zag course or transition to the globular state. These conditions are represented in an amended diagram of HÜRTHLE's (Fig. 149) which takes the above views into account[1]. In the strongly birefringent Q sections the folded primary valence chains are parallel, but are somewhat distorted in the weakly anisotropic I sections. A number of such chains are collected together with the elementary fibrillae and these, in turn, are collected with the myofibrillae surrounded by the sarcoplasm.

[1] MATOLTSY and GERENDÁS (1947) find that the isotropy of the I segment is not caused by desorientation but by an orientated optically negative substance, called N-substance, which locally compensates the positive double refraction of the myofibrillae (GERENDÁS and MATOLTSY, 1947).

It is not clear what the relations are to the septum Z, through which the myofibrillae are supposed to thrust. It is easy to understand, on the other hand, that on both sides slightly birefringent N sections occur, often joining on to the Z stripes, since for this all that would be needed would be less disorientation of the chain in the neighbourhood of Z than in the actual I sections. It is also comprehensible that there should be optical transitions from one section to the other as stated by SCHMIDT (1937a); for the transition from orientated to disorientated regions is less abrupt than as shown in Fig. 149. The intermicellar spaces between the elementary fibrillae certainly belong to the borderlayer system, which is responsible for the chemical processes in muscular contraction, though WEBER has calculated that the globular myogen molecules of the sarcoplasm, the diameter of which, according to SVEDBERG, is 55 Å without the water layer, have no place in it.

SZENT–GYÖRGYI (1946) has recently developed another theory of the microstructure of cross-striated muscle fibres. He states that the myosin of preceding investigators consists of two different proteins, viz., one part actin and three parts myosin s.str., which in the muscle are united to the so-called actomyosin[1]. Myosin s.str. is a fibrillar protein (I.E.P. 5.3) producing highly viscous solutions. Actin, on the other hand, may be obtained as globular protein (I.E.P. 4.7), the solutions of which are of low viscosity; the addition of KCl converts the globular actin to the fibrillar state. For this reason SZENT-GYÖRGYI assumes that actin embodies the contractile principle in muscles. He pictures an actin filament with myosin enveloping it spirally (Fig. 150).

Fig. 150. Model of a single actomyosin spiral (according to SZENT-GYÖRGYI 1946). Black: Strand of actin protein; white: spirally orientated myosin protein.

Looking at the diagram in Fig. 150, we can see how the fibrillar structure may be made up of isotropic and anisotropic segments. SZENT-GYÖRGYI tells us further that the striation of muscle fibres rotated around their longitudinal axis consists, not of cross-segments, but of a spiral with quite flat coils. This would solve the much debated problem of muscular striation.

It is, however, wrong to imagine the orientation of the fibrillar myosin protein as perpendicular to the fibre axis, as in Fig. 150, for which reason SZENT–GYÖRGYI (1945, p. 15) has designed another model in which the myosin particles, though arranged in a spiral round a central actin chain, are not perpendicular to it, but parallel. With this arrangement, however, it is not clear how optically isotropic and anisotropic segments can alternate.

This hypothesis of spirals will probably prove to be untenable since the electron microscope has disclosed a segmentation of submicroscopic protein fibrillae. Submicroscopic segmentation of the protein fibrillae has been found in precipitated blood fibrin (WOLPERS and RUSKA, 1939), ejected trichocysts of *Paramecium* (JAKUS, 1945), the tail of sperms (SCHMITT, 1944), in plasmatic fibrils (MONNÉ, 1946) and

[1] Neither of the two components of actomyosin has been tested by X-ray yet, so that at present we have to rely on the existing records of "myosin" investigations (see Table XXVI).

collagen (Fig. 151a WOLPERS, 1944; SCHMITT, HALL, and JAKUS, 1942, 1943, 1945), the latter having shown that in collagen the period amounts to about 640 Å, which tallies with the X-ray data for long spacing parallel to the fibre axis (BEAR, 1944). The dense segments are marked A, the more transparent, B. Upon artificial elongation the B bands increase in length at the expense of the A segments; the period can be raised to as much as 6000 Å. It is supposed that the polypeptide chains are more

Fig. 151*a*. Collagenous fibrillae from human sinews (after WOLPERS 1944). El. opt. 28000 : 1.

tightly folded or wound up in the A bands than in the B segments and that they partially unwind when artificially elongated. When collagen fibrils are stained with phosphotungstic acid the electron microscope reveals not merely one dark and one bright segment per period but a series of bands (e.g., 5) within the dark segment. Apparently these bands combine preferentially with the phosphotungstic acid. As the dark shading in electron microscopy is caused by the amount of organic matter or by the presence of atoms heavier than carbon, the interpretation of the dark segments of these organic fibrillae is difficult. There might be a denser protein or an accumulation of phosphorus compounds as well.

The electron microscope allows of checking the scheme of Fig. 149 derived from the results of indirect investigation methods. WOLPERS (1944) and HALL, JAKUS and SCHMITT (1946) find in accordance the following micrographs of striped muscle fibres (Fig. 151b): the Q segment is dark and interrupted by a cross band M, whilst the I segment is clear; i.e., there is a denser packing of protein in Q and a much looser arrangement in I, as shown in Fig. 149. The most surprising result is the complete blackness of the Z zone. In this part we must suppose heavy atoms, and as CASPERSSON and THORELL (1941) have found more nucleic acids in the semi-isotropic sections of the fibres, it is likely that phosphorous, besides metallic kations like potassium is accumulated in the Z zone. It might also be possible that the zone Z has a special adsorbing power for heavy metals, since Os-fixation (WOLPERS, 1944) or phospho-

tungstic staining of the fibres have been used in the previous treatment. Fibrils of adductor muscles of the clam of *Venus mercenaria* show a more complicated striation (HALL, JAKUS, and SCHMITT, 1945).

By staining with phosphotungstic acid, HALL, JAKUS, and SCHMITT (1945) were able to detect a like submicroscopic segmentation in the smooth musculature, the fibre period being 725 Å. It would therefore seem that the segmentation of protein

1 μ

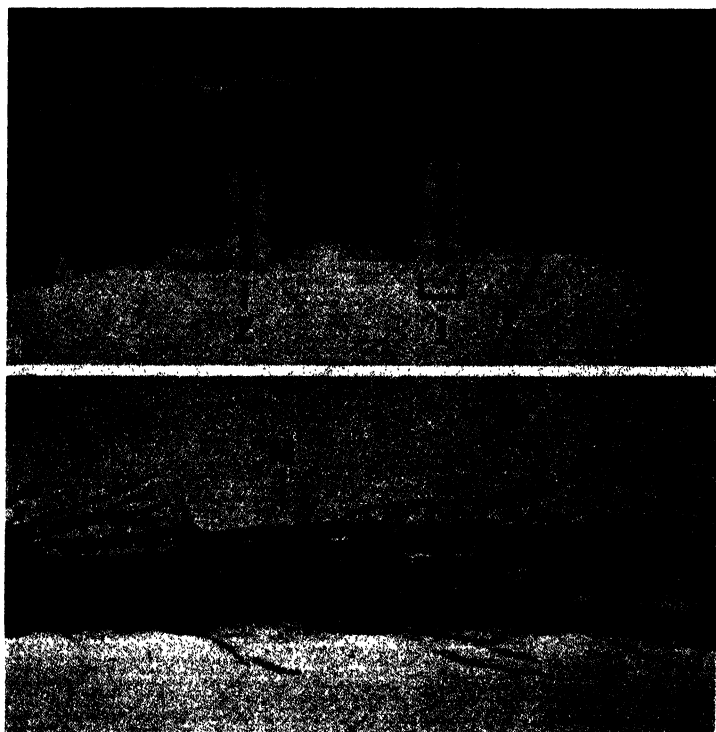



Fig 151*b*. Electron micrograph of striated muscle fibres (HALL, JAKUS and SCHMITT, 1946).

fibrillae is a common property, resulting, as the electron microscope discloses, from the periodic dense and less dense packing of protein or phosphorous substances. The microscopic segmentation of the cross-striated muscles may be regarded as a 50–100-fold enlargement of the observed submicroscopic fibre periodicity.

Mechanism of muscular contraction. There are several ways to attack the important problem of the muscular contraction: thermodynamical, chemical and morphological views may help to find a consistent explanation. Thermodynamics tried to make the desorientation of molecular elements responsible for the liberation of energy when the fibre contracts (cf. BAILEY, 1942). Biochemical investigations show, however, that the energy is liberated by chemical reactions of the protein myosin and the adenosine triphosphate, this nucleotid being dephosphorylated and the liberated phosphoric acid used for the phosphorolysis (s.p. 222) of glycogen. The enzyme adenosine triphosphatase is intimately tied to myosin or represents even a part of this protein molecule (NEEDHAM, 1942 a, b; POTTER, 1944).

In our monograph we have to discuss in the first place the morphological side

of the problem. Fig. 149 represents in diagram the data so far available on the submicroscopic structure of the relaxed muscle. When the muscle contracts, the polypeptide chains coil up somehow. Actually the same thing occurs as in the super-contraction of the keratin chains, with this difference, of course, that in this case the phenomenon is reversible and can be voluntarily induced. A relaxed muscle frozen in liquid air splits up into fibres, whereas a contracted muscle disintegrates into small lumps (MEYER and MARK, 1930). Furthermore, contraction wipes out the X-ray diagram. Roughly speaking, a contracted muscle is amorphous like unextended rubber, whereas in the relaxed state it is crystalline like elongated rubber. Since both the Q and I members shorten under contraction, the coiling of the chains must affect not only the regions of true fibrous structure, but also those comprising disorientated thread molecules.

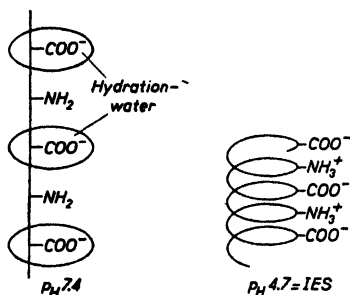



Fig. 152. Contraction of the polypeptide chains in the iso-electric state (IES). (After K. H. MEYER 1929*b*).

Notwithstanding the enormous mass of literature on the physiological processes involved in muscular contraction (VERZÀR, 1943; FAURÉ-FREMIET, 1946), we do not yet know what special process it is that induces the folded myosin chains to supercontract. K. H. MEYER (1929b) suggests that fundamentally it is the mutual repulsion of groups bearing the same charge, e.g., $-COO^-$ groups, which prevents the chains in the relaxed muscle from crumpling. This occurs when the chain is in repose at a p_H of 7.4, viz., in an alkaline medium (see Fig. 152). Now if by some physiological process the p_H of the muscle serum is reduced to 4.7, which corresponds to the isoelectric point of the contracting protein actin, the amino groups become positively charged and the groups with the opposite electric charge are attracted to the point of contact and the chains coil up.

The matter is probably not quite as simple as this, for the charges of the polypeptide thread molecules are not in the primary chain, but at the extremity of the end groups of the side chains. In a later work, MEYER and PICKEN (1937) prove by thermoelastic investigations respecting stretched muscle fibres that, in a state of rest, the polypeptide chains are mobile as in a liquid, whereas fixed bonds are established as soon as the muscle is irritated; thus the molecular frame of the muscles passes from an apparently "fluid" to a solid state. It should be noted that comparability with rubber ceases under these circumstances, for in that material the polyene chains are, conversely, more mobile in the contracted state and are interlocked in the elongated condition. The interesting reaction involved in muscular induration must surely take place between the end groups of neighbouring side chains. It is probable that the discovered reaction of the adenosine triphosphate with the muscle protein consists likewise in a linkage between side chains of the polypeptide with this nucleotid.

This is a good example to demonstrate the consistency of our theory of junctions. Contracted muscle fibres exhibit an extreme gel structure, whilst relaxed fibres show a less tightened structure. It is allowed to make a comparison between the muscle relaxation and the transformation of the plasmagel to the plasmasol in protoplasmic flow, when likewise junctions must be freed to allow a mutual displacement of structural elements.

h. *Myelin Sheath of the Nerves (Neurokeratin)*

The white nerves in Vertebrates consist of a central strand of neurofibrillae enveloped in nerve marrow. Like other fibrous protein substances, optically the neurofibrillae are positively uniaxial, but their birefringence is very weak and is pushed into the background by the very strong anisotropy of the medullary, or myelin sheath (Fig. 153a). The axial string consists mainly of fibrillar proteins and produces rodlet birefringence (BEAR, SCHMITT, and YOUNG, 1937). When heated it shrinks in an axial direction; hence the axial filament shows thermal shortening, like the collagen fibres (SCHMITT and WADE, 1935). The strong birefringence of the sheath is produced by the embedded myelin, which passes out of the nerve marrow upon the addition of water and produces the myelin forms described on page 39. Like the myelinic tubes, the nerve medulla is optically negative as referred to the axial direction. Referred to the radial direction, however, the birefringence is positive. Thus in a cross-section through the nerves the medullary sheath shows a positive cross, while the neurofibrillar plexus appears as isotropic. Since myelin comprises lecithin (Fig. 118), cephalin, cholesterol (Fig. 92) and other anisodiametric optically positive molecules, they must, judging by the birefringence, be orientated in the medullary sheath with the longitudinal axis running radially. Isolated myelinic substances produce X-ray periods corresponding to double the molecular length. There must therefore be bimolecular lipid layers in the nerves. The thicknesses of the layers are given in Table XXV. The average distance between the molecular chains is 4.8 Å (BOEHM, 1933).

TABLE XXV

THICKNESS OF BIMOLECULAR LAYERS OF LIPIDS IN NEURAL MYELIN (AFTER BEAR, PALMER, and SCHMITT, 1941)

Substance	Spacing in Å	
	Determined by X-ray	Calculated from atomic distances
Lecithin	43.4	52
Cephalin	43.8	52
Sphyngomyelin	66.2	65
Kerasin	66.1	64
Phrenosin	50.0	64

The neural medulla does not entirely lose its birefringence when the myelin substances are extracted with fat solvents, but there then appears a negative cross on the cross-section (SCHMIDT, 1937a, b; SCHMITT and BEAR, 1939). This birefringence decreases appreciably when the extracted cross-sections of the nerves are transferred from alcohol to Canada balsam. Thus it is a matter of textural anisotropy, viz., of platelet or laminar birefringence, for the radial direction remains the optical axis, as before the myelin extraction. There must therefore be submicroscopic layers formed out of the neurokeratin, which is to be considered as the frame substance of the medullary sheath. The polypeptide chains of this protein cannot have any preferred orientation, for, if they had, there would be no optical axis in the radial direction. Hence the submicroscopic lamellae of protein are foliate in texture. SCHMIDT (1937a, p. 306, Fig. 80) assumes that there are individual submicroscopic particles of protein which are to some extent independent of each other. This supposi-

tion conflicts with the nature of these insoluble protein lamellae as frame substance, which is more likely to be built up of continuous primary valence chains.

Fig. 153b represents the submicroscopic structure of the medullary sheath according to SCHMIDT (1937b). Lamellae running tangentially from neurokeratin alternate with bimolecular lipid layers. In my opinion the gaps in the protein lamellae represent cut meshes of the protein micellar system through which the myelin

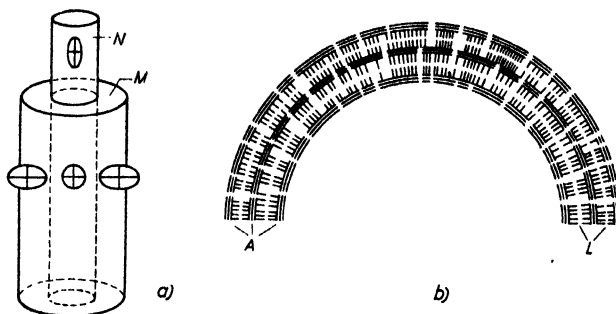



Fig. 153. Fine-structure of medullated nerves. *a*) Optics. N = Neurofibrillar string, positively uniaxial as referred to the axial direction. M = Myelin sheath positively uniaxial as referred to radial direction (after AMBRONN and FREY 1926). *b*) Submicroscopic structure of the medullary sheath (after SCHMIDT 1937b). A = Lamellae of protein. L = Bimolecular lipid layers.

molecules are able to pass. It is difficult to say what the physiological significance of the foliate fine-structure may be. It should anyhow be noted that if this is destroyed, say owing to melting of the myelin substances, so that they are wrenched out of their proper reciprocal positions, the nerves lose their electric conductivity. (Further details in O. SCHMITT, 1936 and O. SCHMIDT, 1942).

SCHMIDT (1937a) detected a similar arrangement of lipid molecules orientated perpendicular to the parallel layers of protein in the outer members of the optic cells in the eyes of Vertebrates, and in this monograph (Fig. 120) such an arrangement has been shown to be probable in the microstructure of the chloroplasts and erythrocytes. It looks, therefore, as if lamellar protein-lipid systems of the kind are fairly common in the submicroscopic morphology of biological objects.

i. *The Protein Frame Substances. Recapitulation*

In a word, the protein frame substances prove to be a surprisingly uniform class of bodies. In all the scleroproteins discussed, the chemistry of which it has been possible to trace in broad outline, and also in coagulated blood fibrin (KATZ and DE ROOY, 1933), polypeptide chains occur whose —CO—CHR—NH— sequences are the members of main valence chains which are combined into long chain molecules of indefinite length, while the side chains R determine the reactivity and associative capacity of neighbouring chains. As all the amino acid residues involved exhibit the α-amino acid configuration, carboxyl, methylene and amino groups alternate regularly in the primary chain. Their interconnection is angled (see Fig. 144) and the length of a three-membered entity, depending on the angle, varies from 2.8 to 3.5 Å, the thickness being approximately 4.6 Å (see Table XXVI). The angles of the zig-zag chain are governed by the character of the side chains. If these are indifferent, as are the methyl groups in silk fibroin, the fibre period remains the length of the member, unaffected by the accompanying groups. As a rule it is

somewhat shorter (blood fibrin, feather keratin, β-keratin) and in collagen (gelatine) it is as short as 2.8 Å. ASTBURY calls these primary chain periods „main-chain spacing" (the fibre period found by X-ray is usually a multiple of this value) and the thickness of the chains „backbone spacing". In the undisturbed state, these two values approximate 3.5 and 4.5 Å respectively. There is also a striking constancy in the, roughly, 10 Å length of the double side chains as well ("side-chain spacing", see Table XXVI) from which fact MARK and PHILIPP (1937) conclude that a general characteristic of the amino residues of the protein frame substances is that spatially they occupy $3\frac{1}{2} \cdot 4\frac{1}{2} \cdot 9\frac{1}{2}$ Å. This generalization, however, goes too far, for the physiologically important distinctions between the various protein frame substances are due to the highly variable character of the side chains, the length of which covers a wide range (see Fig. 90). Thus, according to MEYER and MARK (1930), the side-chain spacing in silk fibroin is only 4.8 Å and, according to KRATKY and KURIYAMA (1931), 6 Å, so the side chains are certainly not long enough to bridge a distance of $9\frac{1}{2}$ Å. It is encouranging enough that the main chain period and the backbone thickness of the various proteins should be so conspicuously uniform; it would be too much to expect all the manifold side chains to be of the same stamp.

TABLE XXVI

DIMENSIONS OF THE AMINO ACID RESIDUES IN THE CHAINS OF THE PROTEIN FRAME SUBSTANCES

	Main Chain Spacing Å	Backbone Spacing Å	Side Chain Spacing Å	Investigator(s)
Silk fibroin	3.5	4.4	4.8	MEYER and MARK, 1930
			6	KRATKY and KURIYAMA, 1931
Blood fibrin	3.35	4.7	10.1	KATZ and DE ROOY, 1933
			11	HERMAN and WORSCHITZ, 1935
Elastoidin	2.9			CHAMPETIER and FAURÉ–FREMIET, 1937
Collagen (gelatine) . .	2.8	4.65	10.0	ASTBURY and ATKIN, 1933
Feather keratin	3.1(—3.3)			ASTBURY and MARWICK, 1932
β-Keratin	3.38	4.65	9.8	ASTBURY, 1933c
α-Keratin	5.03			ASTBURY, 1933c
Myosin (relaxed muscle)	Same as α-keratin			ASTBURY and DICKINSON, 1935b

The side chains are very versatile. In α-keratin and myosin, according to ASTBURY's theory, they permit the main chains to fold and bring about a new chain spacing of about 5 Å. When the side bridges of these folded chains are detached, the primary valence chains suddenly crumple and contract with great force. Cystine-sulphur bridges are supposed to be active in α-keratin, which only relatively drastic treatment can rupture. The side-chain bonds in myosin, on the other hand, are far more labile, with the result that only a slight change in the reaction of the surrounding medium is needed for them to contract, and then the shortened myofibrillae interlink. On account of its lability, myosin has been compared with raw rubber and keratin with vulcanized rubber and, as already stated, the tonofibrillae have been described as "vulcanized" myofibrillae (MARK and PHILIPP, 1937). Convenient as such comparisons undoubtedly may be, they should be applied only with the utmost discretion, particularly as long as our knowledge of myosin is no fuller than it is at the present time.

The typical properties of the polypeptide chains may be said to be the general

tendency to agglomerate into fibrous strands (silk fibroin, blood fibrin) and their widespread power to contract (collagen, keratin, myosin). Thus the very structure of plasmic polypeptides furnishes the fundamental conditions for fibrillar differentiation and contractility.

§ 2. RESERVE SUBSTANCES

a. *Protein Crystalloids*

There is a fundamental difference in many respects between reserve proteins and fibre proteins. First and foremost, the reserve proteins are soluble in water, dilute salt solutions, acids and alkalies, whereas the distinguishing feature of the frame substances is their pronounced insolubility. Reserve proteins frequently tend to crystallize if the solvent is withdrawn in the proper way, as, for instance, by natural means in the formation of aleurone granules owing to the drying up of vacuoles in vegetable storing tissues. Polyhedral, cystallized corpuscles are then formed, different, however, from real crystals in that they are liable to swell and to be stained. NÄGELI (1862) therefore called them crystalloids. Notwithstanding the fact that the term "crystalloid" was later applied by GRAHAM in quite another, and etymologically incorrect, sense to real solutions of substances of low molecular weight, NÄGELI's original definition was retained by botanical cytologists, for to this very day the enclosures of the aleurone granules in the seeds of *Ricinus* (PFEFFER, 1872), *Momordica* (ZIMMERMANN, 1922), *Telfairia* (LEUTHOLD, 1933), etc. are called crystalloids.

Whereas the high polymeric protein frame substances with their polymer-homologous polypeptide chains have no decided molecular weight, defined molecular weights have been found for the reserve proteins by means of SVEDBERG's ultracentrifuge. In spite of the solubility of the reserve proteins, their molecular weights are very high and, strangely enough, show similar values for entirely different proteins. Thus the molecular weight of pepsin, insulin and egg albumin is 34500 (SVEDBERG, 1931) and of casein 32000 (CARPENTER, 1935). In many cases there may be a multiple of these figures, such as 96000 for casein; CARPENTER shows that the association is reversible and depends only upon the concentration of the solution. After the systematic investigation of many proteins, SVEDBERG (1938b) came to the

TABLE XXVII

MOLECULAR WEIGHTS OF GLOBULAR PROTEINS
(AFTER K. H. MEYER, 1940a, p. 409)

Substance	Molecular weight
Lactalbumin α, myoglobin	1 × 17600
Lactoglobulin, ovalbumin, zein, pepsin, insulin	2 × 17600
Serum albumin, CO-haemoglobin, yellow ferment	4 × 17600
Serum globulin	8 × 17600
Edestin, excelsin, phycocyanin, phycoerythrin, catalase	16 × 17600
Haemocyanin (cleavage component), urease	24 × 17600
Haemocyanin (cleavage component)	48 × 17600
Haemocyanin (Calocaris)	96 × 17600
Haemocyanin (Rossia)	192 × 17600
Haemocyanin (Helix pomatia)	384 × 17600

conclusion that a fundamental unit of the molecular weight 17600 is present in all of them and this is found as a whole multiple (rule of multiples) in the various proteins, as Table XXVII will show.

This chart contains none of the protein frame substances dealt with, but only representatives of the reserve proteins, ferments, hormones and pigment proteides.

We learn furthermore from the sedimentation experiments in the ultracentrifuge that the relevant proteins contain globular macromolecules, by which they are distinguished in a striking degree from the chain-like elementary units of the frame proteins. They are therefore compared, as *globular* proteins, to the *fibrillar* scleroproteins.

The crystal lattices of the globular proteins are often pseudo-cubic or pseudohexagonal, of which the occurrence of cubic or rhombohedral crystal shapes in the crystallized reserve proteins of vegetable seeds bears witness. The globular elementary units of the molecular lattice (see p. 20) are so large as to produce a large spaced lattice (Fig. 155a), into the meshes of which swelling agents and dyestuffs can penetrate. The swelling of the rhombohedral protein crystalloids is anisotropic, because, as NÄGELI (1862) had already discovered, it is different parallel to the crystal axis from what it is perpendicular to it. Up to 1939 only seven of all the many crystallizing globular proteins had been identified by X-ray, these being pepsin, insulin, excelsin, lactoglobulin, haemoglobulin, chymotrypsin and tobacco seed globulin (CROWFOOT, 1939, 1941). For, in spite of repeated attempts, it was long before any success crowned the efforts to obtain X-ray photographs of monocrystals of the crystalloids, often well developed crystallographically. Thus, for example, the crystalloids of the seed globulin excelsin of the spruce have threefold symmetry, and those of pepsin hexagonal, but this fact was in no way revealed by the X-ray photograph of a single crystal. On the contrary, until a short while ago all monocrystal photographs of globular proteins, and particularly in the case of the well "crystallized" pepsin (ASTBURY and LOMAX, 1934), have only produced DEBYE–SCHERRER rings with lattice spacings of 4.6 and 11.5 Å, which unexpectedly proved to have the backbone thickness and the sidechain spacing of polypeptide chains. In view of the large molecular weight of the crystallized proteins, it was anticipated that, instead of such spacings, there would be very large periods which would produce the interference dots quite near the centre of the photograph. Although some such large lattice spacings had been found in insulin (CLARK and CORRIGAN, 1932) and in pepsin (FANKUCHEN, 1934), BERNAL and CROWFOOT (1934) were the first to be entirely successful in obtaining monocrystal X-ray diagrams. The secret of their success lay in the fact that they irradiated the pepsin crystalloids (hexagonal bipyramids 2 mm in height) in their mother liquor. They thus discovered a wide-meshed crystal lattice, the elementary regions of which harbour globular macromolecules of about 40000 weight, which figure tallies with the values found in the ultracentrifuge.

The moment the crystalloids are removed from the mother liquor, however, and are exposed to the air, they denature and produce only powder diagrams. Although they retain their crystallographic shape outwardly, apparently the internal regular crystal lattice order can only exist for just so long as the solvent is distributed between the macromolecules.

It would seem that there is some relationship between reserve and frame proteins, notwithstanding the great differences between them in point of solubility and the morphology of the molecular elementary units, for ASTBURY, DICKINSON, and

BAILEY (1935) succeeded in producing filaments and films from the seed globulin edestin and from egg albumin which, when elongated, exhibit the β-keratin type of fibre diagram. ASTBURY therefore assumes the presence of folded polypeptide chains in the crystalloids of the reserve proteins, as represented in Fig. 154. In this way certain self-contained isodiametric areas might be imagined, corresponding approximately to the globular molecules of the reserve proteins, but only capable of existence

```
                                                        ---NH    CO---
                                                            |    |
---NH-CHR-CO    NH-CHR-CO-NH------CO-NH-CHR-CO    NH-CHR-CO---
          |     |
---CC-CHR-NH    CO-CHR-NH-CO------NH-CO-CHR-NH    CO-CHR-NH---
                                            |     |
---NH-CHR-CO    NH-CHR-CO-NH------CO-NH-CHR-CO    NH-CHR-CO---
          |     |
---NH-CHR-NH    CO-CHR-NH-CO------NH-CO-CHR-NH    CO-CHR-NH---
                                            |     |
                                         ---CO    NH---
```

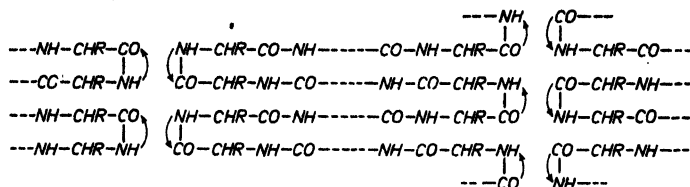



Fig. 154. Molecular structure of a protein crystalloid. The arrows mark the bonds which in „degeneration" to a fibre protein, are resolved to form bridges over the intermediate spaces (which contain solvent) to the neighbouring molecules, by which means straight chains come into existence (after MARK and PHILIPP 1937).

in equilibrium with molecules of the solvent. Where there is denaturation, these loosely-knit complexes would dissociate and long chains would begin to form across the intervening spaces. We can now understand why denatured reserve proteins become partially indigestible, since in this process the polypeptide compounds pass from a loosened soluble form to the insoluble chain form, so hard to hydrolyse, of the frame-protein type (see Fig. 155).

WRINCH (1937) suggests that ring formation of the polypeptide chains may be responsible for the globular shape of the reserve protein molecules. According to her „cyclol theory", the chains would form hexagons by ring folding and forming a bridge bond at the open position between the NH and CO groups. If, by this scheme, six amino acids are assigned to a cyclol six-ring, the result is three regular hexagons arranged trigyrically around a central hexagon. This ternate arrangement falls into line with the trigonal or hexagonal crystal system of the crystallized reserve proteins. As each bridge is formed, alcoholic —C(OH) groups come into existence (see page 106), all the hydroxyls of which lie on the same side of the ring system; this will therefore have a hydrophilic and a hydrophobic side and there will thus be a tendency towards double layer formation. In this view, the protein crystalloids are to be conceived of as packets of double layers of this kind, the hydrophilic planes being responsible for the liability of the crystal lattice to swell.

The great success attending the research into protein crystalloids inspired WRINCH (1941) to propound a „native protein theory", by which plasmic protein is classified as among the globular proteins. In her opinion, plasmic filaments may be pictured as being built up of globular particles. This theory takes no account of the fact that the proteins collected in Table XXVII are all substances which, compared to plasmic protein, are relatively stable and can therefore be peptized to corpuscular disperse sols. This enables them to unite in a crystal lattice, which the labile, reticular plasmic protein is unable to do. Then, muscle protein, with its contractile property, certainly cannot be placed in the category of the globular proteins, since all plasmic contractility is bound up with fibrillar elements. It is therefore wrong, in my opinion, to set up the globular type of proteins as the exemplar of living substance, even as it would be incorrect to take the other extreme, viz., the pronounced fibrillar type

of proteins, as that model. Rather, I believe, should plasmic protein be assigned an intermediate position between the individualized globular molecules and the straight thread molecules which can only exist as chain lattices. The experimental methods at present at our disposal permit exact morphological investigation only of the two extreme cases of reserve and scleroproteins such as those shown in the diagram of Fig. 155, while as yet there has been no satisfactory description of the molecular

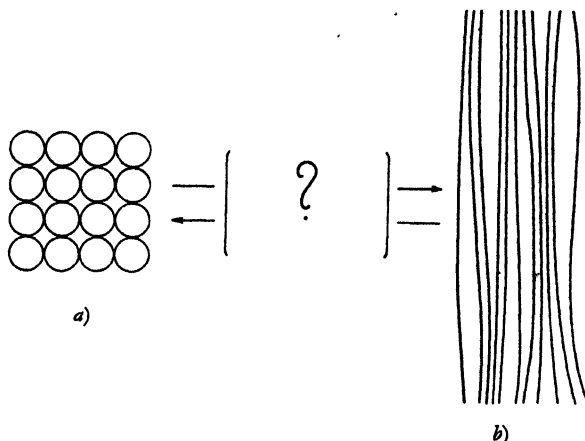



Fig. 155. Model of the fine-structure of protein (after FREY-WYSSLING 1944b). *a*) Lattice of spherical macromolecules (slightly anisotropic or isotropic; highly hydrated); *b*) chain lattice of thread molecules (strongly anisotropic, barely hydrated). The plasmic protein occupies an unknown intermediate position between the two extreme types of structure.

configuration of plasmic protein which, all according to need, may pass either into the fibrillar or into the globular state.

b. *Starch Grains*

Molecular structure of starch. The reserve carbohydrates saccharose, maltose and starch are α-glucosides (see page 42), in contradistinction to the skeleton carbohydrates cellulose, xylane, etc., which are of β-glucosidic structure. Compared to the straight cellulose chains, the glucosane chains with α-1–4 bonds are rather kinked (MEYER and MARK, 1930). The result is that a spatial lattice of such chains must be more flexible and, therefore, is more soluble, as indeed its physiological function as a reserve material requires it to be. Evidently the voids formed by this particular molecular configuration are partly filled with water molecules. Even the simplest α-glucoside, maltose, crystallizes with crystal water, and loosely bound water molecules also play an important part in the crystal lattice of starch. They do not, admittedly, escape from the lattice as easily as from the protein crystalloids, but when grains of starch are crushed, their lattice structure is likewise wrecked as the result of loss of water; they become amorphous, the birefringence and their X-ray diagram (SPONSLER, 1922) vanishing. Hence additive water molecules apparently stabilize the lattice order of starch, as is the case in the reserve proteins. Therefore, until we are able to build up a picture of the distribution of the loosely bound water comprising part of the spatial lattice, we shall have no clearly defined notion of the structure of starch granules in all its details. It is a very difficult problem to solve, because the meshes of the loosely knit lattice labilely joined by water molecules must contain

in addition ambulant water which causes the starch grains to swell and facilitates their intermicellar coloration. There must therefore be all the transitions between lattice-bound, reversibly adsorbed and free water molecules, for the exact characterization of which no entirely satisfactory method has as yet been discovered.

On account of these difficulties, research in respect of structure has hitherto been confined chiefly to that of the elementary units of starch in solution. As already

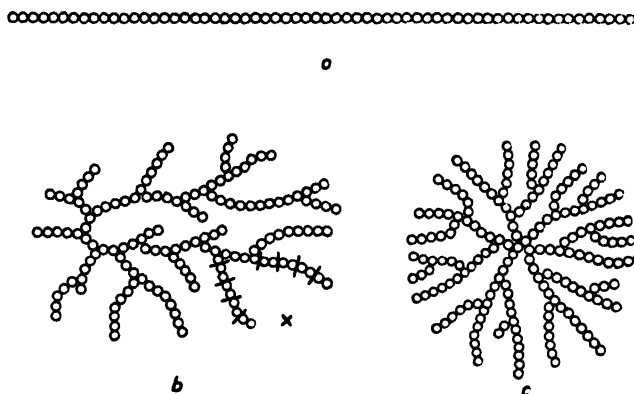



Fig. 156. Diagram of the molecular shapes of glucosanes (after FREY-WYSSLING 1945b). Grape sugar residues represented as small rings: they are far more numerous (degree of polymerization) than as shown here. *a*) Cellulose: unbranched chain up to 1 μ in length. *b*) Starch: branched chain molecule (amylopectin). At x signs of the activity of the sugar-forming amylase: splitting off of the disaccharide maltose. In the absence of the dextrin-forming amylase, degradation ceases if maltose has split off from all the free terminals up to the branching place. *c*) Glycogen: highly branched starch molecule.

stated, these are glucosane chains with α-glucosidic linking (HAWORTH and PEAT, 1926). KARRER and NÄGELI (1921) state that every two glucose residues united to maltose have a certain individuality, from which it may be inferred that the oxygen bridges in the glucosane chains are not of the same value; but probably this is a similar case to the β-glucosidic cellulose chains which, under certain conditions, can be degraded to cellobiose, and therefore likewise to glucose groups of two, despite the fact that all the glucoside bonds in the chain are of the same value. STAUDINGER and HUSEMANN (1937) measured the length of the glucosane chains of starch solutions by the viscometric method. The result was interesting, in that, compared to their degree of polymerization (600–960), the starch chains were found to be surprisingly short, viz., about ten times shorter than the straight cellulose chains with the same number of glucose members. This is endorsed by the fact that neither films can be poured, nor filaments spun from paste. The conclusion those authors draw from this is that the starch chains must be considerably branched (Fig. 156), every second to fourth glucose ring of the main chains carrying a twenty-membered side chain. This would explain why, in the degradation of methyl starch (IRVINE, 1932), considerable quantities of dimethyl glucose (from branching junctions) and tetramethyl glucose (from the end groups of the side chains) are formed in addition to trimethyl glucose. It is also a known fact that branched molecules of homologous series dissolve more readily than do unbranched molecules of the same molecular weight. On the other hand, HAWORTH (1936) and also HASSID and DORE (1937) take into consideration only unbranched, relatively short chains with 26 to 30 glucose residues. According

to STAUDINGER (1937b), the shape of the starch molecule is in between that of the extreme thread-like cellulose molecules and the glycogen molecule, which appears to be a globular glucosane; for the viscosity of glycogen solutions is independent of the degree of polymerization of the glycogen preparations used. In STAUDINGER's view, branching is so marked in this case that every glucose ring of the main chain carries three side chains of 12–18 glucose residues. For reasons of symmetry, however, it scarcely seems possible that the hydroxyls of a main chain would be capable of building glucoside bridges while, according to STAUDINGER's sketch (1937b), the entirely similar hydroxyls of the side chains are unable to do this. It is far more likely that main and side chains become indistinguishable in a macromolecule which, through branching, has become globular. This is illustrated in Fig. 156b, according to K. H. MEYER (1943).

Another possible explanation might be suggested for the shortening of the molecule which may take effect side by side with branching, that being that the glucosane chains buckle or bend (ALSBERG, 1938). Whereas the β-glucosidic bond causes a digyric screw symmetry (Fig. 51 cellulose) which tends to stretch the chain of glucose rings, this symmetrical element disappears in the α-bond and a chain of the kind might quite possibly fold up or roll up (Fig. 51 starch). Repeated bending and simultaneous branching in short side chains would account for the fact that starch molecules retain the rodlet shape even at low degrees of polymerization and therefore obey STAUDINGER's viscosity law.

FREUDENBERG, SCHAAF, DUMPERT and PLOETZ as RUNDLE and EDWARDS argue that the chains of starch molecules may be spiral, six successive glucose rings forming the pitch of a screw. Just as there are H bonds between the neighbouring chain molecules of cellulose, these bonds might occur between contiguous screw pitches of the same chain in the spiral model of the starch molecule. The sixfold glucose rings per convolution are correlated with SCHARDINGER's dextrins, the molecules of which contain 5 to 6 glucose residues (KRATKY and collaborators, 1938). Then, the inside of the hollow cylinders formed by the spiral chain provides the necessary space for the infiltration of iodine in the blue starch reaction. Incidentally, this theory respecting the configuration of the starch molecule encumbers an interpretation of the optical properties of starch grains (FREY–WYSSLING, 1940c). This contradiction might be due to the fact that native starch is different from precipitated starch, which is pure amylose that might have a helical structure when isolated and crystallized.

The chemistry of starch is complicated by the presence in the starch granule of two chemically distinct substances, viz., amylose and amylopectin. Amylose is soluble in hot water and is stained blue by iodine, whereas amylopectin swells in boiling water and gives violet iodine coloration. Thus, when the starch granules form into a paste, amylose goes into solution, while the amylopectin becomes a swollen, insoluble jelly. Neither component exhibits any reducing power upon FEHLING's solution, which signifies that neither contains any free aldehyde groups. K. H. MEYER (1940) has discovered the difference in constitution between amyloses and amylopectin. He states that amyloses consist of unbranched chains, whereas amylopectin is made up of branched chains which together form a gel framework; consequently amyloses are soluble, which amylopectin is not. Their other properties are given in Table XXVIII. The blue starch reaction with iodine is limited to amyloses with crystallized chains, i.e., unbranched chains orientated in parallel (MEYER and BERNFELD, 1941a).

The amylopectin content varies in the different kinds of starch, which accounts for the familiar specific differences between them. Potato starch has a higher amylopectin content than wheat starch (MEYER and BERNFELD, 1941b). Ketan, the starch granules of which are dyed red by iodine, contains only amylopectin of high molecular weight (MEYER and HEINRICH, 1942). Amylopectin possesses weakly acid properties and can therefore be separated by electrophoresis from amyloses, which are absolutely neutral (LAMM, 1937). Presumably the acid groups in amylopectin are responsible for the fact that only basic dyes impart washing-fast staining to the starch grains. SAMEC (1927) says they consist of phosphoric acid. MEYER and MARK questioned in 1930 the existence of phosphoric ester bridges between the glucose chains and nowadays amylopectin (at any rate in maize starch) is declared to be free from phosphoric acid (MEYER and BRENTANO, 1936).

TABLE XXVIII

COMPARISON BETWEEN AMYLOSES AND AMYLOPECTIN

	Amyloses	Amylopectin
Molecular configuration	Unbranched chain	Branched molecule
Molecular weight (osmot.)	10000—100000	50000—1000000
β-Amylase	Complete hydrolysis	Malto dextrin
Pasting	Forms no paste	Forms paste
Films	Solid film	Friable film
Tetramethyl glucose from maize starch	0.31 % nearly nil branches/molecule	3.7 % about 100 branches/molecule

The discovery by HANES (1940) that the enzymatic degradation of starch is a phosphorolysis, and not hydrolysis, invests the phosphorus content of starch grains with a particular significance. This knowledge led to the synthesis of starch in vitro. Starting from phosphorylized glucose, HANES united it with the ferment phosphorylase; when equilibrium sets in between glucose phosphoric acid ester and starch which, owing to the insolubility of the starch, results in its synthesis. HANES' synthesis of starch is the first instance of an artificial build-up of a high polymeric natural product.

The decomposition of starch is a highly complicated process of fermentation (MYRBÄCK, 1938; MYRBÄCK and co-workers, 1942). Amylase, a ferment which decomposes starch, consists of two different ferments, viz., the dextrinogenous α-amylase and the saccharogenous β-amylase. The latter splits off from the aldehyde end of the starch chains twin groups of glucose (Fig. 156b), but is unable to break up the branch junctions of the amylopectin. α-Amylase is able to break down the amylopectin into soluble fragments (dextrins) without at first generating maltose; subsequently saccharification sets in by degrees (MEYER and BERNFELD, 1941c). The branching junctions of the amylopectin are inaccessible to the β-amylase, for, besides the usual 1–4 bond between the glucose residues, there is an additional 6–1 bond passing into the side chain (MYRBÄCK). BARKER, HIRST and YOUNG (1941) state that 6–1 bonds also occur in straight chains. Further details on this matter are to be found in SAMEC (1942, 1943).

The microscopic structure of starch grains. The microscopic structure of starch grains has been dealt with so often and so exhaustively (see BADENHUIZEN's compre-

hensive review 1937) that, to avoid repetition, I shall here touch only on a few points which appear to me of particular importance.

The familiar arrangement in layers of starch grains is brought about, in the unanimous opinion of the majority of investigators from NÄGELI (1858) to our contemporaries, by alternate layers of stronger and weaker refractive power, or containing a smaller or larger percentage of water. Now if a weakly refractive, narrow layer were bordered both inside and outside by neighbours of higher refractivity, it would shine brightly in the microscope at low adjustment and, when the tube is raised, the bright BECKE lines on both sides should pass over into the optically denser layers. This, however, does not take place (FREY-WYSSLING, 1936a, page 287). With pronounced stratification, especially of eccentric starch grains immersed in water (potato, *Pellionia*, etc.), it may be seen distinctly how the BECKE line at the edges of all layers moves *outwards* only when the tube is raised. True, there are cases when a pale lustre can be seen to shift inwards, but on the outside it is always incomparably stronger. From this we may confidently conclude that every layer is more refractive on the inside, the refractive power outwards as a rule diminishing quite gradually, and then suddenly comes up against a layer of higher refractive index at a bound. Thus, in a profile through the grain the refractive power in the various layers is not equally high or low, but there is a continuous decrease centrifugally and discontinuous increase at the outer edges of the layer, as represented in diagram by the serrated line in Fig. 157. The rhythm, therefore, to which the layers are built up cannot be represented as: dense/slightly dense/dense etc., but rather as a gradual impoverishment from dense to slightly dense, coming to a standstill at the edge of the layer, after which, at a stroke, the density leaps up to its initial level. The fact noted by YOUNG (1938) that the layers of the starch grains of *Canna* are more colourable outside than inside is probably due to the looser structure of the outer portions of the layers.

Fig. 157. Microscopic lamination of starch granules. Diagram of the refraction. Abscissa: 1-5 layer edges. Ordinate: Refractive power *n*.

The inference from the foregoing as to the apposition growth of starch grains is that, in the formation of a new layer, the deposition is at first dense, becoming looser little by little until, at a given degree of impoverishment, growth ceases altogether. The sugars consumed have then probably to be made good before the process can start afresh. As SANDE BAKHUIZEN (1925) has pointed out, if external conditions are constant, lamination does not occur, because nutritive material is then always available in the same concentration and, therefore, there is no impoverishment during growth. The same applies to the lamination of cotton fibres, which likewise depends largely upon external conditions of growth, viz., temperature (KERR, 1937), or can, indeed, be prevented altogether by constant exposure to light and the exclusion of fluctuations in temperature (ANDERSON and MOORE, 1937). During their entire growth, the starch grains are enclosed within the amyloplast, which produces them; this stretches very considerably in the process and finally becomes an exceedingly thin, scarcely perceptible pellicle enveloping the grain.

Radial structures have for long been observed in addition to lamination; they take the form of corrosive patterns during the mobilization of the starch in the germinating seeds, or of thin radial cracks. The starch granules have therefore been thought to be of spherite texture. This would seem to receive support from the

optical fact that a positive spherite cross always occurs (see Fig. 68a), provided the amylose chains, like cellulose chains, are optically positive as referred to their long axis.

The starch grains can be split up tangentially and radially by chemical means into minute blocks of 1 μ edge length (HANSON and KATZ, 1934; BADENHUIZEN, 1937) and these particles have been said to be pre-formed elementary units of the starch grain (Fig. 158). Structures of the kind are obtained if starch granules are treated for days on end with 7½ % hydrochloric acid and are then swollen in 2 molar $Ca(NO_3)_2$ (known as "Lintnerization"). HANSON and KATZ suppose that the blocks consist of packets of amyloses and that the swollen intermediate substance is amylopectin. This view is not borne out by the colourability of the substances, since the basic dyestuff, fuchsine, stains the blocks a deep red, whereas the supposed amylopectin intermediate substance remains colourless.

Fig. 158. Microscopic block structure of „lintnerized" wheat starch (after HANSON & KATZ 1934).

Seeing that this block structure is formed only after the application of strong hydrolysis with hydrochloric acid, the view of pre-formation is hardly tenable; it is more likely to be a case of hydrolysis patterns (FREY–WYSSLING, 1936a, p. 290). The kind of partitioning of objects made up of high-polymeric chain molecules has likewise been observed in the production of chemical cross-sections through cellulose fibres with sulphuric acid (KELANEY and SEARLE, 1930), the decomposition of cotton into "dermatosomes" by hydrochloric acid (FARR and ECKERSON, 1934) and in the decomposition of muscle fibres in acid alcohol (SCHMIDT, 1937a, p. 180). Thus in all these cases hydrolytic reagents are necessary to produce the reported dissociations. Considering how sensitive highpolymeric main valence chains with glucoside or peptide bonds are to hydrolysis, it must be deemed out of the question that the reagents used would merely have a dissolving effect; they surely cannot fail to induce break-down and decomposition. Cellulose chains, for instance, are broken down by 1 n HCl (= 3.6 %) at 53° C. in six hours from 1660 to 445 degree of polymerization (STAUDINGER and SORKIN, 1937a) and amylose chains are shortened in only 3½ minutes by 2 n HCl from 940 degree of polymerization to one-fifth their length (STAUDINGER and HUSEMANN, 1937). Nor is it surprising that this hydrolytic degradation should take place above all across the particular texture (fibrous or spherite) since the hydrolysis occurs perpendicularly to the alignment of the thread molecules. The partitioning parallel to the axis of orientation need not necessarily be of hydrolytic nature; it is as likely to take place in a less drastic, physical way (radial cleavage, cracks due to drying, fibrillation through swelling), for in this direction there are chiefly secondary and not primary valencies that have to be overcome.

A question which it is difficult to answer is why the hydrolysis should set in with such conspicuous rhythm in the case of these microscopically homogeneous structures. Were the microscopic segments formed to correspond to the chain lengths of the macromolecules, as STAUDINGER, STAUDINGER, and SAUTER (1937) assume that they do in the laminate break-down of synthetically produced polyoxymethylene crystals, mechanical, instead of chemical, cleavability perpendi-

cularly to the crystal axis should occur, but this is lacking in fibres; therefore, any such interpretation would not apply to natural fibres. Another possible explanation is that maybe the submicroscopic capillary system of the objects in question, corresponding to the hydrolysis pattern, is periodically fine and coarse. Without any such auxiliary hypotheses, however, it is not difficult to suppose that in the hydrolytic break-down of fibrous or spherite structures fragments of the same kind are produced, just as, in the mechanical pulverization of crystals or glass, only segments or splinters of approximately the same size split off, this size having nothing to do with the structural elementary units, but depending solely upon the method of comminution applied. Macroscopically as well this rule rests on objects of entirely uniform structure; thus, when ice is broken up, a perfectly homogeneous slab of ice may split up into floes of equal proportions, the size of which is by no means predetermined. Under certain circumstances and, of course, to an enormously enlarged scale, the pattern of the floes may be strikingly reminiscent of the block structure represented in Fig. 158. In the opinion of BADENHUIZEN (1938) the "blocks" certainly are not predetermined by the structure of the starch granules.

The submicroscopic structure of starch grains. So far, X-ray analysis — so important an element in the investigation of the submicroscopic structure of birefringent objects — has contributed but little to the elucidation of the structure of starch granules. KATZ and his collaborators have, however, been able to establish that different kinds of starch produce X-ray spectra peculiar to themselves. For example, the gramineous starch of wheat, rice, maize and oats produces what is known as an A spectrum, whereas potato starch has a B spectrum, and both, when formed into a paste, produce a third, called the V spectrum. Starches with a B spectrum have been converted at higher temperatures to the A kind (KATZ and DERKSEN, 1933); it has also been shown that the V spectrum reverts to a B spectrum in the so-called retrogradation of paste, in which process the quantity of bound water plays a certain part. Thus the following conversions may be observed in wheat starch which is pasted up and then retrogresses: A $\rightarrow$ V $\rightarrow$ B.

Several investigators (SPONSLER, 1923; v. NARAY–SZABO, 1928) have attempted to deduce the size of the elementary cell of crystallized starch. BEAR and DEXTER (1941) find for B starch an orthorhombic cell with a volume of 930 Å^3 and for A starch a triclinic cell with 843 Å^3 volume. This is much more then the cellulose cell which occupies only 670 Å^3. This proves that besides glucose residues water molecules are enclosed in the cell. But these results are doubtful, as starch produces only powder diagrams, i.e., DEBYE–SCHERRER rings, and no one has yet succeeded in making starch preparations of higher orientation and thus in obtaining point diagrams. Hence no estimates as to lattice spacing will suffice to decide on the structure of the starch grain so long as crystallographic directions are not known. For instance, polarization patterns make it fairly evident that the amylose chains are orientated radially, but the X-ray method supplies no proof of this (Fig. 159a). Recently KREGER (1946) has succeeded in irradiating only part of the large starch grains of *Phajus grandifolius* by a special micromethod. In this way he gets a fibre pattern, which allows of calculating the cell of B starch more exactly. Unfortunately no data are obtained for the main chain spacing. KREGER therefore assumes it to be about 10.4 Å as in cellulose. In this way he calculates an orthorhombic cell of B starch with a : b : c = 9.04 : 10.40 : 15.50 Å, i.e. a volume of 1785 Å^3, giving space to 8 glucose residues and 8 molecules of water.

As long as the important fibre spacing b cannot be measured this result is uncertain.

It is nevertheless possible to build up a clear picture of the submicroscopic structure of starch granules without the help of X-ray data. This may be based on evidence produced by another reserve carbohydrate, viz., mannane, which is obtained from the tuber of *Amorphophallus konjak* and is marketed in Japan under the name of "Konyaku". The technical commercial product consists of irregularly

a *b*

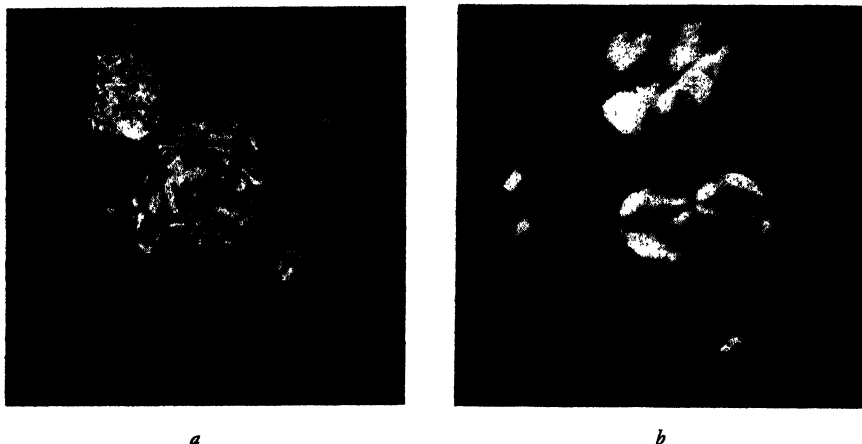



Fig. 159. Mannane grains of konyaku (*Amorphophallus konjak*) in the polarizing microscope. Embedding medium *a*) xylene; *b*) water; there is the transient appearance of a spherite cross.

bordered granules which light up in a quite irregular manner between crossed nicols and reveal no ordered structure (Fig. 159a). But when these grains of konjak are observed in water, they are seen to undergo a remarkable change. Under the very eyes of the observer, they swell and assume a spherotexture, exhibiting a positive spherite cross (Fig. 159b). After a time the appearance becomes fainter and eventually vanishes altogether, because the swelling is not limited, but continues until solution takes place.

This phenomenon may be interpreted as follows: The mannane chains, which are comparable to the amylose chains, in the dry konjak grain (which it is best to examine in a hydrophobic embedding medium, such as xylene or Canada balsam) are arranged in bundles that accumulate in the grain without any defined orientation. This explains why the polarizing picture is irregular. As soon as water is added to these chains, however, they are hydrated, become mutually mobile and align themselves radially and this results in a spherotexture. The conditions are much the same as those in myelin figures (see Figs. 47 and 48), except that hydration is not limited to one hydrophilic pole of the molecule, but encompasses the entire mannane chain and ultimately spreads to such an extent that the individual thread molecules become independent of each other and go into solution.

The stage of voluntary spherite formation is comparable to the structure of starch grains. The starch molecules are obviously also fixed in a radial direction by water of hydration. In this case, however, the water does not function as a solvent, but in some way participates, as a loosely bound constituent, in the build-up of the spatial lattice. The starch chains are far more highly polymeric than the molecules of

konjak mannanes. Notwithstanding this, the amyloses are soluble and, if the starch grain seems nevertheless to swell only to a limited degree, there must be some particular hindrance to swelling. This is probably to be found in the amylopectin, the glucosane chains of which are interlinked. There is good reason to believe that these amylopectinous linkages occur in each individual layer of the starch grains in the inner, denser and more refractive portions and that independent, amylose chains are accumulated in the outer, looser portions of the layers. JALOVECZKY (1942) states that the lamellae containing amylopectin are isotropic, whereas those containing amylose are anisotropic and can be stained.

Fig. 160. Diagram of the submicroscopic structure of a layer of starch granule. *a* Outer portion looser, less refractive, with little interlinking: *i* inner portion denser, more refractive, more closely interlinked.

It has been suggested that all the amylopectin is localized in the outermost marginal layer of starch grains, which is resistant when they are pasted up. It would seem more probable, however, that the starch is liable to every conceivable transition from the easily soluble amyloses to the virtually insoluble constituent of the amylopectin, which resists even enzymatic degradation. Thus it might be supposed that amylopectins occur in the denser portions of all the layers, though not in the same degree as in the insoluble outside layer which resists when starch is pasted up. On this assumption the submicroscopic structure of starch grains might be represented as in Fig. 160.

This diagram takes into account the following observed facts: The density and refractive power at the core of a layer diminish gradually towards the outer regions and then increase suddenly at the boundary of the layer. Solubility within the layer is not everywhere equal. The water of constitution between the chains is partially bound in the form of a lattice and is partially present as mobile swelling water. The swelling maximum of the granules is governed by the linkage of the chains. Adjacent layers have coalesced. The structure is wide-meshed and porous, to which are due the colourability and rodlet birefringence (SPEICH, 1941). In the process of pasting up the loosely linked, or unlinked glucosan chains go into solution as amylose, whereas the strongly linked amylopectin chains agglutinate throughout the paste. Minor specific or individual variations in linkage may be responsible for the peculiar resistance of different kinds of starch, or of different grains within the same kind of starch. For instance, if we take the grains of potato starch, the peripheral layer of which is so resistant to enzymes that some of them may pass unaffected through the intestines (WEICHSEL,

Fig. 161. Rodlet birefringence curves of potato starch (SPEICH 1941). A in lipophilic liquids; B in aldehydes; C in monovalent alcohols (except ethanol); D in water, enthanol, glycol, glycerol and their mixtures.

1936), it must be supposed that this is a case of an exceedingly close interlinking of the glucosan chains in the enveloping outer layer. These distinctions disappear in the degradation of starch by acid, as all glucosidic linkages are then dissolved hydrolytically.

We do not yet know whether the meshwork of Fig. 160 consists of individual molecular threads (molecular frame), or of bundles of chains (micellar frame). The changeability of the X-ray diagram reported by KATZ would seem to point to the former for, if this is really caused by different embedding of water in the crystal lattice, the water must be embedded, not between bundles of molecules (intermicellarly), but between the individual chains, i.e., intramicellarly, or rather, intermolecularly.

The analysis of the rodlet birefringence (SPEICH, 1941) likewise supports this view. Series of aldehydes, monovalent alcohols and polyvalent alcohols (glycol, glycerol) inclusively water give three different curves of rodlet birefringence (Fig. 161), disclosing different intrinsic double refractions in these three groups of liquids. This is due to the different interaction of these compounds with the starch chains. Lipophilic liquids (amylbromide, xylene, toluene, benzene, chlorobenzene, bromobenzene, and α-bromonaphtene) do not penetrate into the starch grain, because they have no affinity to starch; the double refraction therefore does not change in series of lipophilic liquids with increasing refractive power.

Of all the theories so far developed respecting the structure of starch granules, that propounded by A. MEYER (1895) comes nearest to the views set forth here. True, instead of his dendritic branching, we assume all-round interlinking, and the dimensions of the structure are reduced by some orders of magnitude to the submicroscopic, or even to the molecular.

RETROSPECT

A revolutionary fact which emerged from the synthesis of organic compounds was that, in chemistry, there is no fundamental difference between living and inanimate matter. The complicated process of metabolism is not controlled by some special vitalistic principle, but has its being in the wonderful co-ordination of innumerable reactions taking place in every conceivable direction, each and all, as individual reactions, being accessible to causal investigation. Yet no broad mechanistic interpretation can account for their delicately attuned correlative harmony and their purposiveness. Now, morphological formation in the submicroscopic world presents an exactly similar case. Whoever had expected to find special biological formative principles, alien to the inanimate world, in these invisible regions, is doomed to as great a disappointment by the results of research into natural substances of high molecular weight as was at one time suffered by the believers in mysterious life forces which alone were deemed capable of building up organic compounds. The formative forces in protoplasm and its derivatives are no different from those operating within inanimate organic Nature. There is no evidence of the existence of novel formative principles beside the atomic valence and molecular cohesive forces in their various patterns. This need cause no surprise if it be remembered that, in the molecular world, the chemical and formative properties merge into each other, as it were. In that realm, chemistry and morphology become inseparably one, since every morphological change which a molecule undergoes inevitably involves chemical changes. Thus all metabolic processes run parallel to changes in molecular form. For this reason substance and form are closely interrelated, not only in the inanimate world, where every compound can be clearly classified by its molecular or crystal structure, but in living matter as well. Hence the essential difference between the morphology of the animate and that of the inanimate world, as defined in the Preface, has no place in the theory of submicroscopic morphology.

Just as organic chemistry grew out of inorganic chemistry and has its roots in the fundamental principles of the latter, so should biomorphology be considered, merely as a highly developed system, evolved from molecular and micellar morphology to the shaping of cells and organisms. Admittedly, only the first step in this development at present lends itself to deductive reasoning, viz., the transition from molecular to micellar morphology, which the modern evidence respecting the structure of highly polymeric thread molecules has made possible.

There are two guiding principles, of the utmost importance to biomorphology, which are already recognizable in the configuration of chain molecules. They are: 1. The principle of repetition, which is the foundation of all lattice structures and of every segmentation and 2. The principle of specificity. The first principle is represented, on the one hand, by the ever-recurring members of the chain

(intramolecular spacing) and, on the other, by the assemblage into a lattice pattern of kindred chains (intermolecular spacing), as e.g.: frame substances, reserve substances, lipidic layers. Only if all the members of a certain kind of chain are of exactly the same structure can legitimate intermolecular repetition take place. This law does not as a rule apply to polypeptide chains, as their side groups are often of totally different structure. This deprives them of the capacity to crystallize and, we find the second principle holding sway, i.e., the capacity of otherwise similar molecular elementary units to assume a specific arrangement. As yet we do not know how the visible specific forms grow out of this specificity, but doubtless causal relations do exist between molecular morphology and the operation of vital processes, as clearly foreshadowed by enzyme chemistry and the asymmetrical synthesis of organic compounds.

A problem no less difficult than causal morphological development is that of the molecular morphology of heredity; for, assuming that every kind of visible form owes its origin to particular configurations of concrete hereditary entities which cannot arise spontaneously, then their complicated structures must be constantly reproducing their like. Although the multiplication of the mysterious virus molecules presents some analogy to this, we have nothing to go upon to build up a clear picture of the automatic duplication of those complicated structures, the genes.

For the present, submicroscopic morphology has been successful only in so far as specificity is ignored, but within this modest sphere the knowledge acquired is most significant. The substratum in which life is inherent is not a dispersoid with individual particles or ultramicrons; it possesses a structure. Its active groups, which control metabolism and development, are juxtaposed in a given order. They are not intermingled by mere laws of chance and Brownian molecular movement; the fact is rather that the chain molecules arrange themselves into a delicate, very plastic and flexible molecular frame, actuated, as it were, by a purposeful, co-ordinative impulse. No more than leaves, blown by autumnal winds from the twig and fluttering helplessly in the air, are able to assimilate for the parent tree, can independent, ambulant, reactive molecules take part in any organized work. It is not surprising, therefore, that the active groups of the enzymes should only be capable of acting in symplex association with a carrier of a given structure. For, orderly biological processes are unthinkable without presupposing structure, and it is therefore out of the question that any living constituent of protoplasm could consist of structureless, fluid, independently displaceable particles. It is for this reason that colloid chemistry, based, as it is, upon the dispersoid principle, has thrown so little light upon the submicroscopic structure of protoplasm. For the cell certainly is not a pouch filled with ultramicrons suspended in a fluid, whirling about haphazardly and in confusion; it is, on the contrary, a wonderful system, the intrinsic structure of which, could it but be seen, would assuredly fill every observer with an enthusiasm equal to that which microscopic cytomorphology inspires.

In the inanimate world, crystallization will at times produce structures from an amorphous mass; but the structures of living protoplasm cannot be spontaneously generated from unformed solutions because, complicated and delicately inter-adjusted as they are, they can only actualize in contact with already existing structures. Hence the supreme axiom of cytology, viz., that all cells derive from their like, applies equally, though in a wider sense, to invisible, submicroscopic cytogenesis:

STRUCTURA OMNIS E STRUCTURA

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